THE SYNTHESIS OF SINGLE-ISOMER CYCLODEXTRINS FOR THE ENANTIOMERIC SEPARATION OF SULFOXIDES AND SULFOXIMINES

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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

In Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

May 2002

ABSTRACT

Chiral sulfoxides belong to the class of chiral organosulfur compounds that are widely used in asymmetric synthesis. Their application as chiral synthons has become a well established and reliable strategy. This is mainly due to their availability and high asymmetric induction exerted by the chiral sulfinyl group. Equally important in making effective use of these reagents is the ability to efficiently separate these compounds and to be able to determine the enantiomeric excess of one enantiomer over the other.

In this research effort, the investigator proposes the synthesis of single isomer cyclodextrins in order to develop an effective method to determine enantiomeric excess in mixtures of sulfoxide and sulfoximine enantiomers. These single-isomer cyclodextrins are employed as chiral resolving agents in capillary electrophoresis for the enantiomeric separation of chiral sulfoxide derivatives.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF NMR DATA

ACKNOWLEDGMENTS

I would like to express my heartfelt appreciation to my advisor, Dr. Thomas Green for all the wonderful advice, knowledge and guidance. Without his tremendous insight and knowledge, this research would not have been possible. I want to thank the wonderful faculty and staff of the department of chemistry for providing a friendly and stimulating academic environment. I will never forget your kindness and friendship.

I also would like to thank my undergraduate advisor at Hope College, Dr. Michael Silver. He gave me my love for chemical research and discovery and taught me that often research chemistry is as close to art as it is to science.

I want to thank the medicinal chemistry group at Pfizer Inc., for offering me the opportunity to start a new and exciting chapter in my life, and chemical career.

Most of all, I want to thank my parents, Herman and Netty Smeets, for having been there, always. For having taught me about perseverance, integrity and compassion. One could not ask for better role-models. I also want to thank my sister Nicole and her husband Marcel, for always being only a phone call away.

Most importantly, I want to thank my wonderful wife, Shelli, for putting up with me throughout this experience, for "braving" Alaska with me, and for all she means to me. I love you.

Marc I. Smeets

Fairbanks, Alaska

December 30, 2001

Science is nothing but trained and organized common sense, differing from the latter only as a veteran may differ from a raw recruit: and its methods differ from those of common sense only as far as the guardsman's cut and thrust differ from the manner in which a savage wields his club

Thomas Henry Huxley, 1893

CHAPTER 1. INTRODUCTION

1.1 A Brief History of Chiral Sulfoxides and Sulfoximines Separations

Since the 1960s we have seen a great surge in interest in chiral sulfur compounds and their chemistry[1]. Initially, chiral sulfur compounds served as model compounds in studies on the mechanism and stereochemistry of nucleophilic substitution at sulfur's center. It did not take long for chemists to realize that chiral sulfur compounds are of great value in asymmetric synthesis. Many reactions may be efficiently stereo-controlled by chiral sulfur species which later are easily removable, under mild conditions, by reductive or eliminative methods. There is great potential for enantiomerically pure sulfur reagents to transmit chirality to other centers but in order to do so we need to develop equally powerful methods for separating and quantifying these enantiomeric compounds. These new chiral compounds have led to the rapid development of new materials in fields like pharmaceutical chemistry, natural products and agrochemicals.

The focus here lies on chiral sulfoxides and sulfoximines. These molecular species belong to the class of chiral organosulfur compounds which are most widely used in asymmetric synthesis. Their application as chiral synthons has now become a well-established and reliable strategy. This is mainly due to their availability and high asymmetric induction exerted by the chiral sulfinyl group. Important classes of sulfoxides in asymmetric synthesis include alkylarylsulfoxides, dialkylsulfoxides, allylsulfoxides, vinylsulfoxides and β-oxosulfoxides.

Traditionally, chiral sulfoxides are resolved by means of either shift reagent nuclear magnetic resonance[2] (NMR) or high performance liquid chromatography[3]. Effective chiral sulfoxide separations have been achieved by means of custom pack HPLC. In this technique, liquid chromatography columns are packed with a custom designed and synthesized stationary phase, which is appropriate for a specific family of compounds. This technique has shown to be relatively successful. Matlin et al. published data[4] which showed excellent resolution between the enantiomers of a wide range of different sulfoxides, using custom phase polysaccharide HPLC columns. The use of polysaccharide phases in chiral separations had been reported previously[5] and although these columns are often well suited for chiral separations, there are also numerous downfalls for using this technique. Custom packing of HPLC columns often leads to poor reproducibility which is a major obstacle, especially in high-throughput, industrial settings where good reproducibility can mean the difference between a product being able to go to market or having to be reworked and further purified.

Poor reproducibility is caused by several factors. The major problem stems from the fact that these custom phases need to be applied to a silica stationary phase. First this silica gel needs to be chemically adapted so as to ensure proper coating by the custom phase. Once this is achieved, it becomes very important that the silica is homogeneously coated with the custom stationary phase. When the stationary phase is synthesized, one has to ensure equivalent chemistry each time a column is prepared. Since most of these syntheses are relatively simple, this is often not a concern. This is the part which often leads to poor reproducibility. Over the length of the LC column there will be a density

gradient of both the silica and the amount of custom phase coating on the silica particle. When packing the columns, one has to be very careful in avoiding the formation of possible voids. It has also been suggested[6] that silica with larger pore sizes (500, 1000Å) needs to be packed very carefully, or the particles can actually collapse due to pressure buildup inside the column.

Another major drawback to using HPLC analyses techniques is the fact that very large amounts of mobile phase are consumed. Often times, mobile phase is recycled, but this leads to definite contamination issues and is often not desirable in an analytical laboratory setting. One advantage though, of the HPLC technique, is the excellent sensitivity and the wide range of detector modes available. This technique is still by far the most popular technique available for resolving sulfoxide enantiomers. This is mainly due to the fact that HPLC is a mature technique which is really a gold standard in the industrial analytical field.

The second method for determining enantiomeric excess in sulfoxide mixtures is the shift reagent NMR technique. An increasing number of reports are being published on the NMR methods that enable the elucidation of the absolute configuration of chiral secondary alcohols, amines, and carboxylic acids[7]. It has not been until relatively recently that this technique has been employed in resolving chiral sulfoxides[8]. This technique, although relatively sensitive, requires the use of expensive chiral shift reagents and tends to be very time consuming. It has been reported recently that 9-Antryl-1,1,1-trifluoroethanol, (S)-α-methoxyphenylacetic acid, and (R)-(-)-N-(3,5-dinitrobenzoyl)-α-phenylethylamine[9] are excellent shift reagents for deducing the absolute configuration of

sulfoxides. One major obstacle in using this technique is that, when determining the enantiomeric excess of a compound which exhibits complex spin-coupling, it often becomes difficult to interpret the data and line broadening often becomes an issue, which in turn leads to loss in resolution.

The two techniques described above are the main ways with which we can currently elucidate and resolve chiral sulfoxides, but there has been a trend both in industry, as well as in academia, to develop better techniques to analyze these synthetically interesting species. One of these techniques is capillary electrophoresis using cyclodextrins as chiral selector reagents. In the next section I will outline why this technique is an excellent alternative to the techniques discussed in this section.

1.2 Stimulus for Synthesizing Single Isomer Functionalized Cyclodextrins

It quickly becomes apparent why sulfoxides are of great synthetic value and interest. It also is quite apparent that current techniques for enantiomerically separating sulfoxide products are quite limited in their scope. In this research thesis I would like to discuss a relatively new technique, in the quest for rapid, effective separation of sulfoxides, through their amination to sulfoximines[10].

The technique of capillary electrophoresis is based on charge separation by means of chiral selectors, which are dissolved in the mobile phase. These chiral selectors can be a variety of different functionalities (crown ethers, cyclodextrins etc.). Our research will focus on cyclodextrins. Figure 1 shows how different analytes complex with the charged cyclodextrins. The different enantiomers will have different complexation times and

energies, due to differences in analyte-cyclodextrin interactions, and hence will reside inside the charged cyclodextrins for a different amount of time. This in turn leads to resolution between the two enantiomers.

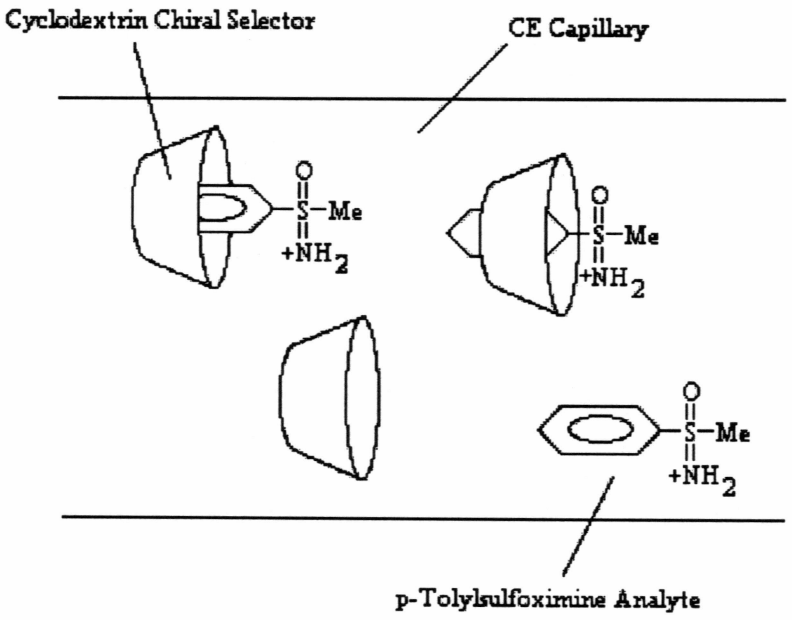


Fig. 1 Analyte-Cyclodextrin interaction

The reason we are interested in using single isomer cyclodextrins, as opposed to mixed substituted cyclodextrins, is because this will give us improved resolution[12]. Once we establish that we are dealing with only one level of substitution, we can then ensure that the cyclodextrin is also a single isomer. It is important to note that the reason we are interested in charged cyclodextrins, as opposed to neutral cyclodextrins, is because charged cyclodextrins give us a larger elution window when analyzing a sample. When using a neutral cylodextrin, the elution window is only as wide as the resolution between

the electro-osmotic flow (EOF, the bulk movement of the mobile phase, towards the detector) and the specific retention time of the sample. This elution window becomes much wider when using charged cyclodextrins. Figure 2 depicts this improvement in resolution when using a charged cyclodextrin system.

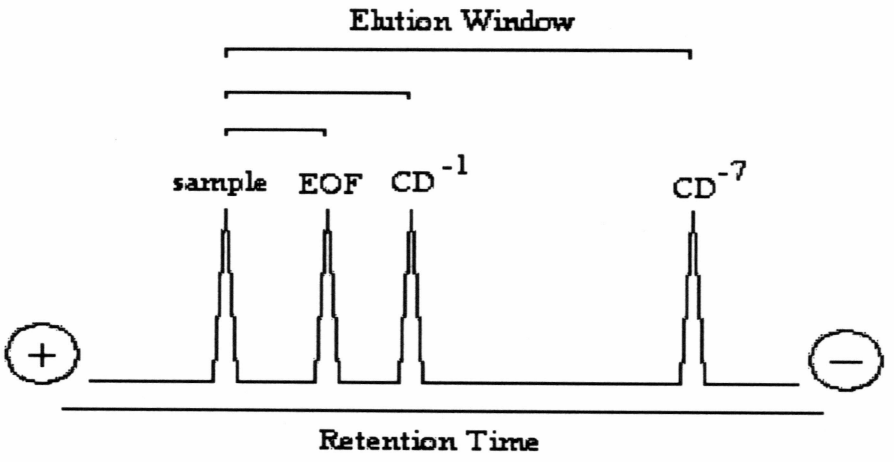


Fig. 2 Elution window size versus charge on the cyclodextrin

Figure 1 shows that the best resolution is obtained when using a cyclodextrin with a large negative charge. At some point though, the retention times become very long which limits us to the most effective cyclodextrin charges as being -6 or -7. The ultimate stimulus of this research is to create a novel and effective way to resolve sulfoxides and sulfoximines, but the first phase of this research will mainly involve developing and perfecting synthetic methods for creating these functionalized cyclodextrins. Once the single isomer cyclodextrin has been synthesized and isolated, the second phase of the research will involve the separation of sulfoxides and sulfoximines.

The third and future phase of this research would use these data sets to evaluate theoretical models and to establish models for how charged and uncharged cyclodextrins interact with each other and the analytes. Current theoretical models assume very little interaction between the charged and uncharged cyclodextrins, so future models, based on our research, might be a great improvement and determine whether or not these assumptions are correct. The third phase of this research project is beyond the scope of what I will be involved in and will be a continuation of my research by future graduate students in the Green group.

Currently, only a relatively limited number of functionalized cyclodextrins are commercially available and these cyclodextrins are not single isomers but instead mixtures of substituted products. These varying degrees of substitution will lead to poor chiral resolution[12]. By developing novel, single isomer cyclodextrins, we can determine if these new structures are more effective in separating charged and uncharged species. We will synthesize several single isomer cyclodextrins in which the primary hydroxyl groups are fully substituted with some form of functionalized anionic group while the secondary hydroxyl groups are methylated.

As stated previously, we will focus on sulfoxides because of a variety of reasons. It is known that sulfoxides form strong inclusion complexes with cyclodextrins[13] and there are a wide variety of convenient ways to synthesize racemic and enantiomerically enriched sulfoxides. Most importantly, sulfoxides can be derivatized to charged sulfoximines with complete retention of stereochemistry[10].

1.3 Cyclodextrin Capillary Electrophoresis

Although high performance liquid chromatography (HPLC) has been used for over 20 years in chiral separations, the use of capillary electrophoresis (CE) for chiral separation has grown rapidly only in the last 10 years. Compared to HPLC, chiral separation using capillary electrophoresis has the advantages of higher separation efficiency, enhanced speed of analysis, and flexibility of rapid incorporation of various chiral selectors. This research will focus on developing new compounds in the cyclodextrin family, which may be used as effective chiral selectors, for separating enantiomeric sulfoxides and sulfoximines. These syntheses are often tedious, multi-step syntheses and much time is needed to develop a new compound, but since the capillary volume in capillary electrophoresis is small (<50 μl), only very small amounts of chiral selector are needed to develop an effective separation system. On average the chiral selectors are present at only 500 μM to 5 mM concentrations. This means that when we synthesize, for example, 1 gram of cyclodextrin compound, this will be sufficient for performing many (>> 100) separations. Hence the process of developing and synthesizing these multi-step compounds becomes economically very feasible and it becomes apparent that this research is not only a purely academic endeavor but also can be of industrial interest.

The most commonly employed chiral selectors for small molecules are cyclodextrins. They offer high selectivity for a wide range of chiral molecules[14] and are available in modified forms, both charged and neutral. To this point, the use of mixed cyclodextrins as chiral selectors in capillary electrophoresis has been used successfully in

a variety of chiral and achiral separations[15]. This mode of analytical separation is commonly referred to as cyclodextrin capillary electrophoresis. This technique is a very powerful one, mainly due to the combination of all the strengths of capillary electrophoresis with the wide range of molecular selectivities which are offered by cyclodextrins with different fucntionalities. The problem with commercially available charged cyclodextrins is that they vary greatly in their degree of substitution and are sometimes poorly characterized as to degree of substitution. By using single isomer charged cyclodextrins, one expects to obtain better resolution and one can develop better predictive models, since the cyclodextrin structural entity is exactly known and the system is much more simple as compared to mixed cyclodextrin systems, where a variety of cyclodextrins are used at once. This is of interest to those who want to develop separation models, in which case one needs to determine such factors as intrinsic selectivity, equilibruim constants and electrophoretic mobilities. For these determinations, single isomer cyclodextrins are also preferred because charged cyclodextrin mixtures may contain many individual cyclodextrins with different selectivities, which often will result in poor resolution. Vigh refers to this effect as "parasitic complexation" between the cyclodextrin and the inserting, guest species[14].

Vigh and coworkers have synthesized a family of single isomer anionic cyclodextrins, including heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin, hepta-6-sulfato-β-cyclodextrin and heptakis (2,3-diacetyl-6-sulfato)-β-cyclodextrin[12]. All of these compounds were successfully employed in the separation of both chiral and achiral, small molecules. Kraus developed and synthesized the route to single isomer

anionic heptakis(6-O-carboxymethyl-2,3-di-O-methyl)-β-cyclodextrin[16], which in turn was used by Culha et al.[17] in the capillary electrophorertic separation of a variety of coumpounds. Other commonly employed anionic cyclodextrins for chiral analyses include sulfopropylether and sulfobutylether cyclodextrins. These latter mixtures have always been employed as mixtures of isomers, with varying degrees of substitution. Our main focus was on the synthesis of a single isomer, carboxylated cyclodextrin, but we also started working on the synthesis of a butylsulfated cyclodextrin which contains a 4-carbon "spacer" between the sulfate group and the cyclodextrin hydrophobic center.

CHAPTER 2. EXPERIMENTAL SECTION

2.1 Materials and Equipment

The capillary electrophoresis system used is a Beckman Inc. P/ACE 2000 system. The Beckman is connected to a Pentium III PC by means of a National Instruments 6035E data acquisition card. This 12-bit card can operate up to 200 kHz and provides us with 10 analog inputs and 2 analog outputs, in addition to 10 DI/O channels. I used a National Instruments BNC 2110 connector block to connect the capillary electrophoresis instrument to the DAQ card. I wrote the programs to interface the DAQ card and the CE system using the National Instruments LABView 6.i programming language. Data was collected at 20 Hz unless noted otherwise. Data was saved in a .dat format and incorporated into Microsoft Excel 2000 for graphing and manipulation.
All chemicals were purchased from Aldrich Chemical Company unless noted otherwise. I used the department's Varian Mercury 300 MHz NMR for structural identification. The NMR runs under a VNMR environment. Some structural identification was performed using the chemistry department's HP5890 Series II/HP5972 GC/MS instrument.

2.2 Synthesis of O-Mesitylsulfonylhydroxylamine

The synthesis of this powerful aminating reagent consists of a two-step synthesis. There are two common ways of synthesizing the hydroxylamine moiety (**4**, Fig. 3):
1) Carpino's method[18] which uses t-butyl N-hydroxycarbamate (**1**; Fig. 3)

2) Zinner's method[19] using ethyl acetohydroxamate (**2**; Fig. 3).

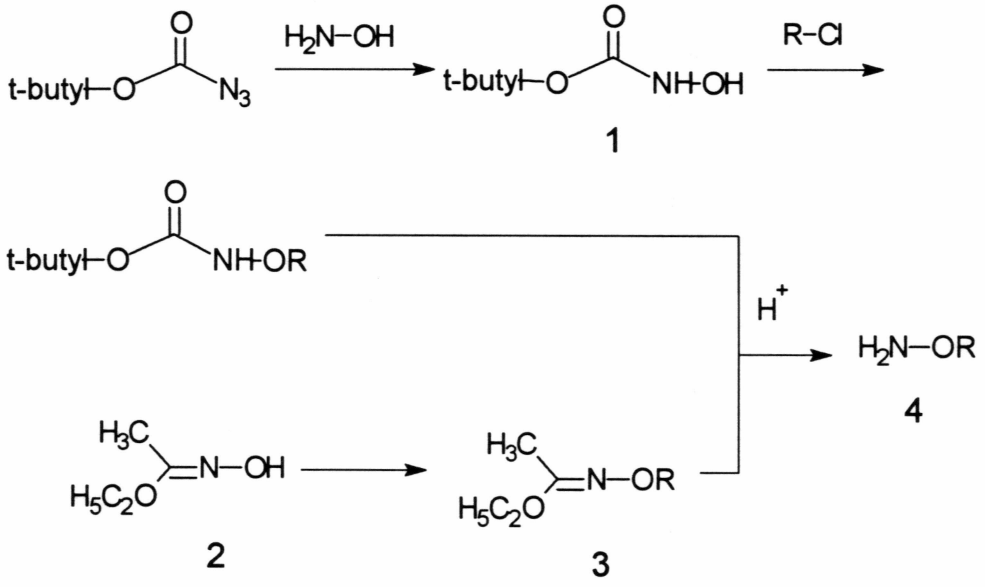


Fig. 3 Synthesis of hydroxylamine aminating reagent

The hydrolysis proceeds easiest using 70% perchloric acid but there have been reports in the literature where trifluoroacetic acid and hydrochloric acid were used succesfully[20].

Other interesting methods have been reported for the synthesis of the hydroxylamine reagent[21]. One such method involves treatment of hydroxylamine hydrochloride with potassium cynanate which produces O-aminocarbonylhydroxylamine in 32-35% yields. The problem is that aminocarbonylhydroxamic acid is also produced as a competing reaction. Ultimately we decided to use the Zinner varation which is a two step route which leads to O-mesitylsulfonylhydroxylamine (**4**; Fig. 4).

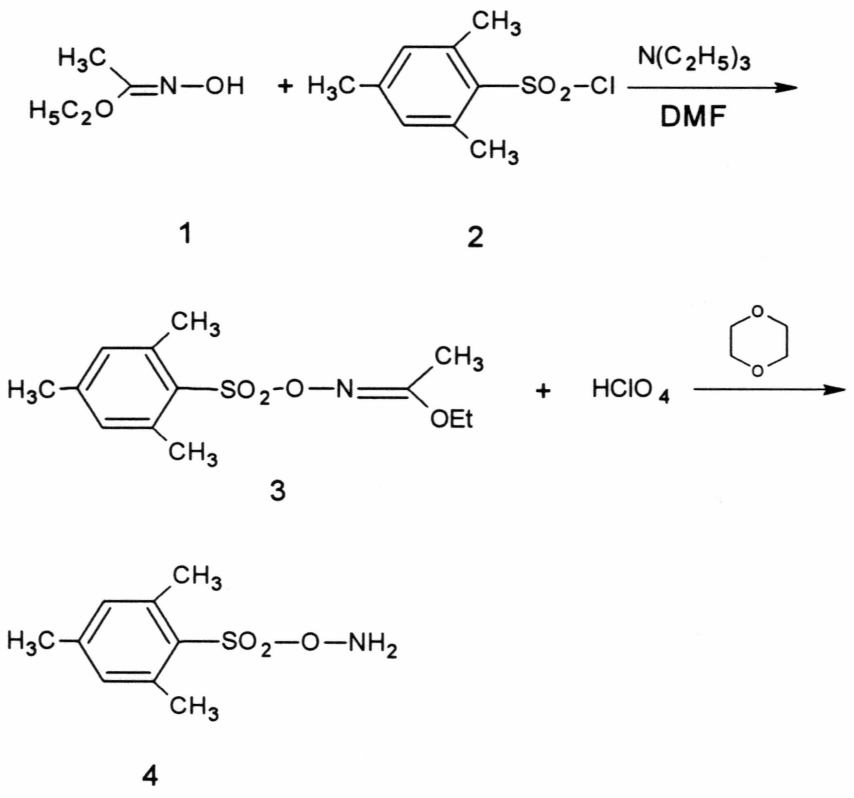


Fig. 4 Synthesis of O-Mesitylsulfonylhydroxylamine

The first step in this synthesis involves the formation of ethyl O-(mesitylenesulfonyl)acetohydroxamate (**3**, Fig. 4). Powdered mesitylenesulfonyl chloride (**2**, Fig. 4; 10 g, 46 mmoles) was added to a solution of ethyl acetohydroxamate (**1**, Fig. 4; 4.7 g, 46 mmoles) and triethylamine (7 ml) in DMF (12.5 ml) in small portions (approximately 500 mg portions) over a period of about 20 minutes with stirring at approximately 0°C. The reaction was performed under an argon atmosphere. Precipitation of triethylammonium mesitylenesulfonate is observed after the first addition of

mesitylenesulfonyl chloride. After complete addition of mesitylenesulfonyl chloride, the solution was of a cloudy, yellow-orange color. Upon complete addition, stirring of the reaction mixture was continued for an additional 20 minutes at 0°C. The reaction mixture was then poured into 200 ml of water (0°C), a white, crystalline precipitated formed and was filtered out. The solid was washed several times with distilled, cold water after which the white, crystalline solid was dissolved in diethyl ether (50 ml). The ether was dried (Na_2SO_4), and removed under vacuum to give a slightly yellowish solid. This solid was recrystallized with petroleum ether and allowed to slowly precipitate out at 4°C, overnight. The next day the crystalline, white-yellowish solid was collected and the mother liquor was concentrated, seeded with a crystal of the above obtained solid, and once again cooled overnight to give more product. All collected fractions were combined to give a yield of approximately 22%. The product was characterized by ^{1}H NMR (NMR-1) (δ=1.1 (t, 3H), 2.0(s, 3H), 2.2(s, 3H), 2.6(s, 6H), 3.8(q, 2H), 7.0(s, 2H)) and melting point (lit.[20] 53-55°C).

The product of the above reaction was used in the synthesis of O-mesityl sulfonylhydroxylamine (**4**, Fig. 4). Ethyl O-(mesitylenesulfonyl)- acetohydroxamate (**3**, Fig. 4, 0.36 grams, 1.3 mmoles) was dissolved in approximately 3 ml of 1,4-dioxane, to give a white, creamy colored solution. Perchloric acid was added by syringe in small increments over 10 minutes. Upon each addition, the reaction vessel was lowered into an ice bath and a white crystalline solid formed. After an additional reaction time of 10 minutes, the mixture was poured into 100 ml of water and immediately a white solid precipitated. The solid was vaccuum filtered and washed with some water (0°C). The

solid dissolved in approximately 5 ml of diethyl ether. The ether layer was dried (K_2CO_3). The diethyl ether was then pipetted into cold petroleum ether (50 ml). A white precipitate formed over 1-2 minutes. This white, crystalline solid was filtered under vaccuum and characterized by ^1H NMR (NMR-2). The solid was stored at 4°C, under argon, because of the compounds instability. The reaction ultimately gave a yield of 33%.

2.3 Synthesis of Racemic Methyl p-Tolylsulfoximine

In order to effectively analyze a sulfoxide species by cyclodextrin capillary electrophoresis, we need to transform it into a charged species, while retaining the stereochemistry. It was Johnson and Kirchoff [10] who showed that indeed, the stereocenter is retained when transforming the sulfoxide into a sulfoximine.

This is of great importance to our research for if the stereocenter of the sulfoxide is not retained upon derivatization, our research efforts would be futile, since derivatization the reaction itself would change the stereochemistry. The following figure (Fig. 5) depicts our synthetic route for creating racemic sulfoxides. We use the powerful aminating reagent which was described in section 2.2 to derivatize the methyl p-tolylsulfoxide to a sulfoximine species.

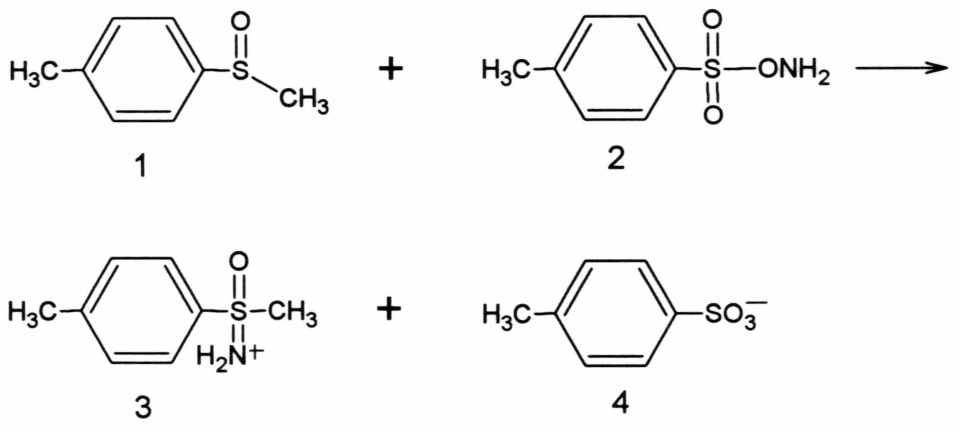


Fig. 5 Synthesis of racemic methyl p-tolylsulfoximine

For the first step in this reaction scheme, racemic methyl p-tolylsulfoxide (**1**, Fig. 5, 36.3 mg, 0.24 mmoles) was added to 1.0 ml of dry (K_2CO_3) dichloromethane. This solution is stirred and 78.3 mg (0.36 mmoles) of O-mesitylsulfonylhydroxylamine (**2**, Fig. 4) is added in increments over approximately 5 minutes. The reaction mixture is stirred for 3 hours after which the white, cloudy solution is pumped to dryness to give a white solid (yield: 78%). An NMR was taken in d6- DMSO (NMR-3).

It is interesting to note that the NH_2^+ protons (**3**, Fig. 5) should absorb between 6-8.5 ppm, in ^1H NMR. These protons undergo slow exchange and tend to be very broad peaks. When expanding the NMR in the 6-9 ppm region, we see a very broad peak. It is believed that this represents the NH_2^+ protons.

2.4 Synthesis of Single Isomer Heptakis(6-O-carboxymethyl-2,3-dimethyl)cyclo-maltoheptose

This synthesis was developed by Kraus [16]. The overall synthetic route is shown below in Fig. 6.

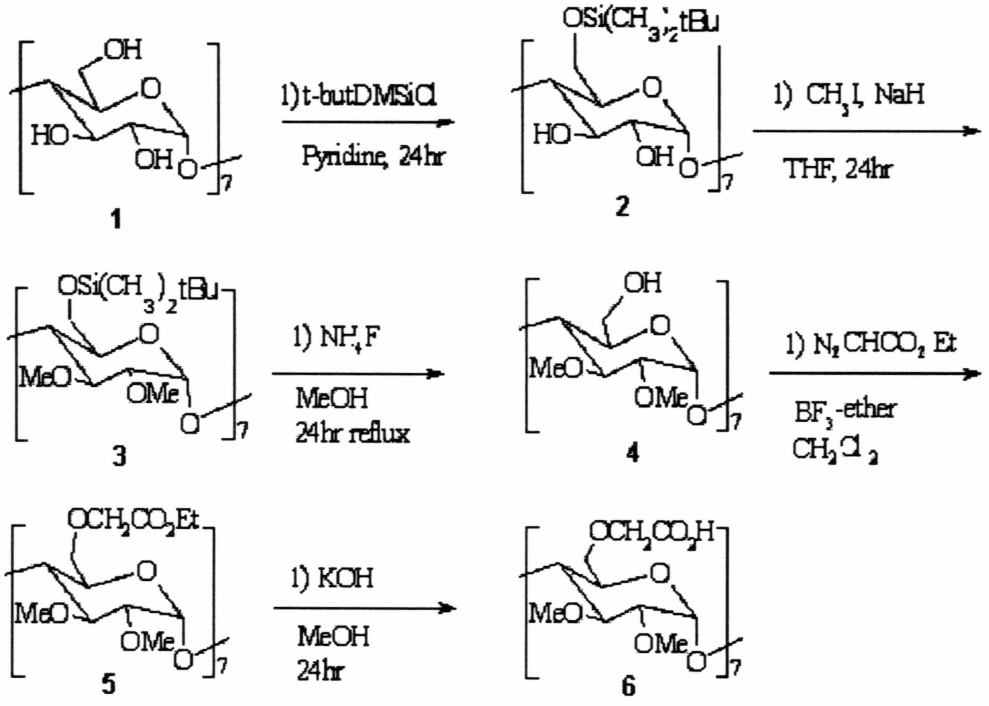


Fig. 6 Synthesis of heptakis(6-O-carboxymethyl-2,3-dimethyl)cyclomaltoheptose.

The starting material for this synthesis is dry, beta-cyclodextrin (Aldrich). This cyclodextrin consists of seven cyclomaltoheptose repeating units. The first step involves the synthesis of heptakis (6-O-tert-butyldimethylsilyl)cyclomaltoheptose (**2**, Fig. 6). The same procedure as described and published by Fugedi et al.[22] was followed except that 10.5 equivalents of tert-butyldimethylsilylchloride was used, instead of 7.7 equivalents.

This was done to ensure protection of all 7 primary alcohols. In order to ensure proper drying of the starting material, β-cylcodextrin (4.54 g, 4 mmoles) was placed in a crystallizing dish in the oven at 70°C for 24 hours. After this period, the dry β-cyclodextrin was dissolved in dry pyridine (60 ml). The solution was cooled to 0°C and a solution of tert-butyldimethylsilylchloride (6.45 g, 42.8 mmol) in dry pyridine (40 ml) was added drop-wise, over a 15 minute period. The mixture was kept at 0°C for 3 hours and then at room temperature overnight. The following day, the solution was poured into 700 ml of ice water, while stirring. A fluffy white precipitate formed, which floated to the top of the beaker. The precipitate was vacuum filtered, washed with 600 ml of water and then dried, at room temperature, overnight under vacuum. The product (**2**, Fig. 6), a white powdery solid, could be used without further purification and was characterized by ^{13}C-NMR (NMR-4) in $CDCl_3$. The reaction yield was 93% (7.61 grams). The NMR assignments were compared to and matched with Fugedi's assignments[22] (δ = 101.8 (C-1), 81.6 (C-4), 73.5 (C-2, C-3), 72.5 (C-5), 61.6 (C-6), 25.9 ($CC(CH_3)_3$), 18.2 ($CC(CH_3)_3$), $CDCl_3$)

The next step involves the synthesis of heptakis(6-O-tert-butyldimethylsilyl-2,3-di-O-methyl)cyclomaltoheptose (**3**, Fig. 6). For this step we used the procedures as described and published by Takeo et al.[23]. Heptakis (6-O-tert-butyldimethylsilyl) cyclomaltoheptose (**2**, Fig. 6) (3.00 g, 1.55 mmol) was dissolved in dry, sure-seal bottled tetrahydrofuran (THF) (70 ml). Sodium hydride, NaH (2.80 g, 60% in mineral oil), was washed (hexane), vacuum filtered and stored under an argon atmosphere. Using a vacuum, the remaining hexane was pumped off the NaH. The dry NaH was added in

several portions to the dry THF solution of **2** (Fig. 6). The solution was stirred under argon for 2 hours at room temperature. The reaction was then cooled again to 0°C and methyl iodide (7.20 ml, 115 mmol) was added dropwise over 15 minutes. This solution was then stirred at room temperature overnight. The next day we obtained a orange, creamy solution to which small portions of methanol were added, at 0°C, to decompose any unreacted sodium hydride. The solvents were then removed by rotary-evaporation at 60°C, to obtain a light brown residue. This oily residue was dissolved in 5 ml of dichloromethane and subjected to flash chromatography on silica gel. The solvent gradient went from 4:1 hexane/ethyl acetate to 2:1 hexane/ethyl acetate. The main reason for this column separation is to separate the product from any leftover mineral oil which was not removed before the NaH addition. When this reaction scheme was repeated, I replaced this step with a simple separation, using a separatory funnel. The mineral oil and the reaction mixture form two very distinct, separate layers. Upon removal of the solvents by reduced pressure pumping, we obtained a white, crystalline solid (4.89 g, 76% yield). ^{13}C NMR analysis showed this to be heptakis(6-O-tert-butyldimethylsilyl-2,3-di-O-methyl)cyclomaltoheptose (**3**, Fig. 6). ^{13}C-NMR was taken in $CDCl_3$ (NMR-5) and assignments matched those of Takeo et al.[23] (δ=98.1 (C-1), 82.2 (C-4), 82.0 (C-2), 78.6 (C-3), 72.1 (C-5), 62.3 (C-6), 61.5, 58.6 (2 OCH_3), 25.9 ($CC(CH_3)_3$), 18.3 ($CC(CH_3)_3$), $CDCl_3$)

Step 3 involves the synthesis of heptakis(2,3-di-O-methyl)cyclomaltoheptose (**4**, Fig. 6), This synthesis involves the O-desilylation of heptakis(6-O-tert-butyldimethyl-silyl-2,3-di-O-methyl)cyclomaltoheptose (**3**, Fig. 6) using the methodology developed by

Zhang and Robins[24]. Heptakis(6-O-tert-butyldimethylsilyl-2,3-di-O-methyl)-cyclomaltoheptose (**3**, Fig. 6) (4.80 g, 2.25 mmol) was dissolved in 250 ml of methanol. Ammonium fluoride, NH_4F (5.15 g, 0.139 mmol) was added and the solution was refluxed overnight under a positive argon pressure atmosphere. The methanol was removed by rotary evaporation at 60°C, followed by drying under vacuum at room temperature for 15 minutes. Dichloromethane (200 ml) was added and the solution was suction filtered to remove insoluble NH_4F.

The dichloromethane was removed by rotary evaporation at 60°C to obtain a slightly yellow, crystalline solid. ^{13}C-NMR data (NMR-6) matched Zhang's assignments[24] (δ= 100.4 (C-1), 84.2 (C-4), 83.3 (C-2), 80.4 (C-3), 74.8 (C-5), 63.0 (C-6), 63.6, 61.2 (2 OCH_3), D_2O).

The next step involves the synthesis of heptakis(6-O-ethoxycarbonylmethyl-2,3-di-O-methyl)cyclomaltoheptose (**5**, Fig. 6). The procedures developed by Kraus et al.[16] were employed. Heptakis(2,3-di-O-methyl)cyclomaltoheptose (**4**, Fig. 6) (2.20 g, 1.65 mmol) was dissolved in dry dichloromethane (36 ml), ethyl diazoacetate (1.7 ml, 1.60 mmol) was added under argon atmosphere. BF_3 diethyl etherate (200 µl, 1.58 mmol) was added and bubble formation was observed in the reaction solution, as nitrogen gas was released. The reaction solution was stirred at room temperature for 24 hours. After this period of time, 15 ml of a 2% $NaHCO_3$ solution was added and the mixture was stirred for approximately 5 minutes. The dichloromethane layer was dried ($MgSO_4$), concentrated under a vacuum, to give an extremely sticky, yellow-white, fibrous looking solid. This solid was re-dissolved in dichloromethane and subjected to column

chromatography (SiGel). Approximately the first 50 ml fraction consisted of 100% chloroform, after which a 25:1 chloroform-methanol mixture was employed. Approximately sixty, 5 ml fractions were collected.

The following bands were observed;

Fraction #	Band Color	Solvent Ratio (Chloroform:Methanol)
1/30	Colorless	25-to-1
31/35	Yellow	25-to-1
36/38	Colorless	25-to-1
39/45	Yellow	25-to-1
46/55	Colorless	25-to-1
55/60	Orange	100% MeOH

Table I, Column fractions of heptakis(6-O-ethoxycarbonylmethyl-2,3-di-O-methyl)cyclomaltoheptose (**5,** Fig. 6) separation.

Fractions 39-45 contained product (TLC) and were concentrated under vacuum. The product, a white-to-slightly yellow compound, was isolated and analyzed by ^{13}C-NMR (NMR-7). It seemed possible that those later fractions contained products with lesser degrees of substitution (hexakis, pentakis etc.) and that there also was some unreacted ethyl diazoacetate (this would explain the orange color of the last several fractions). The ^{13}C NMR matched to the assignments made by Kraus[16] and it seemed very likely that we indeed isolated a single isomer compound. Only 1.12 gram of product was recovered which translated to approximately 35% yield. Assignments were made as follows: δ=98.1 (C-1), 81.8 (C-2), 81.5 (C-3), 80.3 (C-4), 70.7 (C-5), 68.4 (C-6), 61.2, 58.2 (2 OCH_3), 70.4 (OCH_2), 60.2 (CH_2CH_3), 13.9 (CH_2CH_3), 169.9 (CO-O), D_2O).

The final step in this synthesis involves the synthesis of heptakis(6-O-carboxymethyl-2,3-di-O-methyl)cyclomaltoheptose (**6**, Fig. 6). Once again, we followed the procedures as described by Kraus[16] Heptakis(6-O-ethoxycarbonylmethyl-2,3-di-O-methyl)cyclomaltoheptose (**5**, Fig. 6) was dissolved in CH_3OH (5 ml) and then 2.6 ml of 1 N aqueous KOH solution was added. This reaction mixture was stirred at room temperature for 24 hours. The methanol was removed by rotary evaporation and water (5 ml) was added. This mixture was then applied to a column of Dowex 50W-X4 (6 ml, washed with 1N HCl prior to use) until the eluant was neutral using pH paper. The water was removed by rotary evaporation at 70°C and the product was then dried under vacuum at room temperature, overnight, to obtain heptakis(6-O-carboxymethyl-2,3-di-O-methyl)cyclomaltoheptose (**6**, Fig. 6) (yield: 41%). The product was characterized by ^{13}C-NMR (D_2O). (NMR-8) Assignments matched those published by Kraus[16] (δ=94.7 (C-1), 78.6 (C-2), 77.8 (C-3), 74.9 (C-4), 68.2 (C-5), 65.7 (C-6), 57.0, 55.8 (2 OCH_3), 67.8 (OCH_2), 169.9 (CO-O), D_2O).

2.5 Synthesis of Single Isomer Heptakis(6-butylsultone-2,3-dimethyl)cyclomaltoheptose

By changing the functionalities on the cyclodextrin, one can alter its chemical behavior and this leads to different separation mechanisms when using the single isomer cyclodextrin in capillary electrophoretic applications. Figure 7 shows the proposed

synthetic plan for the synthesis of heptakis(6-sulfobutyl-2,3-dimethyl)cyclo-maltoheptose.

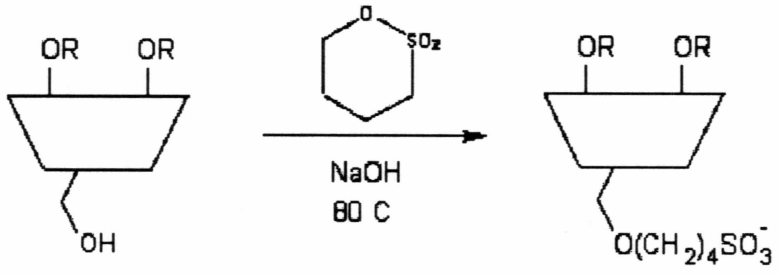
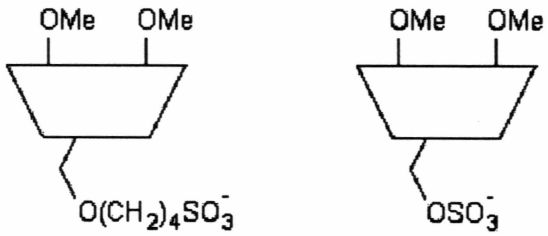


Fig. 7 Synthesis of heptakis(6-butylsultone-2,3-dimethyl)cyclomaltoheptose.

In 1998, Vigh et al. published and interesting article[25] on the synthesis of heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin. They showed that this functionalized cyclodextrin was very effiective in the enantiomeric separation of a wide variety of phenyl-functionalized organics. Our motivation to synthesize heptakis(6-sulfobutyl-2,3-dimethyl)cyclomaltoheptose is based on this same priniciple but our functionalized cyclodextrin will contain carbon atom "spacers" before the sulfato functionality (Fig. 8).



Green et al. Vigh et al.

Fig. 8 Comparison of Green versus Vigh sulfato-functionalized cyclodextrin.

Vigh used HF to deprotect the silated alcohol after the methylation step of the Williamson ether synthesis. We have used NH_4F which is much milder but we have observed problems with yield in this conversion. I would consider using HF in future experiments, although the safety risks associated with using HF are greater and extreme caution must be observed.

Stella et al. have studied sulfobutyl functionalized cyclodextrins. They filed a patent[26] in 1994 regarding the use of their cyclodextrins in enhancing the aqueous solubility of pharmaceutical drugs. It appeared that certain sulfobutyl-functionalized cyclodextrins might increase drug delivery rates of specific pharmaceuticals to some parts of the body. We have used some of this patent information to develop an effective synthetic route to the heptakis(6-butylsultone-2,3-dimethyl)cyclomaltoheptose moiety. The methylated, deprotected cyclodextrin (**4**, Fig. 6, 100 mg, 0.07 mmoles) is dissolved in 2 ml of 1 N NaOH solution. 6 ml of water is added to dissolve all the cyclodextrin.

The 1,4-butylsultone (24 mg, 0.21 mmoles) is added in a 3:1 sultone:CD molar excess. This mixture is then stirred at room temperature for 24 hours. Although we performed this synthesis, at the time of writing the product was still in the identification phase. We did perform an indirect detection experiment (see Chapter 3) which gave us some interesting data.

2.6 Conditions for the Cyclodrextrin Capillary Electrophoresis System

In this section I will discuss a variety of different, yet related issues, regarding the capillary electrophoresis system and the conditions under which it was run.
I will also discuss some of the details of the LABView programming used to log our data. It was this same program which was later modified and used in Dr. Green's CE-Laser Induced Fluorescence system. Finally, I will discuss sample and buffer preparation. In chapter 3, I will discuss the actual observed separations.

2.6.1 System Design

The Beckman P/ACE 2000 capillary electrophoresis instrument was used in all of our cyclodextrin capillary electrophoresis experiments. This system was designed by Beckman in the late 1980s but still functions quite effectively. All measurements were taken at either 214 nm or 254 nm. We used a 50 cm x 75 μm, bare fused silica column. Injections were made in pressure injection mode and not voltage mode. Pressure injections were 6 seconds in duration. The column was prepared by running a 1% NaOH solutions through it for 5 minutes, followed by 10 minutes of rinsing with high purity

water (>13 MΩ resistance). The capillary electrophoresis system uses two platinum electrodes that are periodically cleaned using methanol and Kimberly wipes. The electrodes are always kept in water overnight to prevent deposits of ionic compounds the surface of the electrodes. Separations were done anywhere between 11 kV and 25 kV (<100 μA current). In order to ensure proper column and system conditions, we purchased a standard solution mixture kit from Beckman. This kit contains standardized buffer solution and a sample mixture which consists of benzoic acid, p-hydroxybenzoic acid, p-hydroxyphenylacetic acid in a standardized EDTA solution. The sample is analyzed using a boric acid buffer, which is also provided in the kit.

The CE instrument was hooked up to an integrator plotter, but since it is much more preferable to save data in a digital format, LABView was used to create a ".dat" file format (see appendix 2). The LABView program which was created is based on what is called a "state machine". It is a two step process in which first the header and data labels of the file are created after which the program collects data at a data rate which can be set by the user. The data is then either displayed on the computer screen, saved to a data file, or both. The front panel of the program contains an on/off switch to start or terminate data collection, a switch to start or stop the saving of data and a display for showing the chromatogram. It is important to note that the data acquisition card (6053E series DAQ card from National Instruments) is set to have full, 12-bit resolution between 0 and 1 volts of instrument output. This means that the card can accurately break up a 1 volt data range into 4096 (2^{12}) parts which means that the optimum resolution would be 244 μV signals.

The CE LABView program front panel also contains a sampling frequency control and two text input controls to create remarks, which are saved with the data to the file. The time is tracked and reset during each new injection. This means that the time and voltage signal are saved to file. This makes graphing "time" versus "response", a very simple task using Microsoft Excel. The program also tracks data sampling rate versus actual program execution loop rate and saves this loop execution rate to file for each program loop iteration. The user controlled sampling rate divided by actual loop execution rate should be very close to 1, if not, the timing of the program is not correct. Since LABView corrects for PC processor speed, this ratio always seems to be within 0.5% of 1.0 and hence more than acceptable. In general, our data collection rate was 20 Hz, which lead to approximately 20-60 data points per peak. The CE instrument was connected to the DAQ card through a National Instruments BNC-2110 BNC connector block, which in turn was connected to the DAQ card inside the PC. The CE sample tray contains vials containing 1% NaOH for column conditioning, distilled water for rinsing, buffer inlet and buffer outlet solution (with the buffer containing the cyclodextrin), sample solution and a waste vial.

2.6.2 Sample and Buffer Preparation

There were four separate sample and buffer preparations; preparation of the Beckman standard sample, preparation of the commercial sulfated cyclodextrin buffer solution, preparation of the single isomer heptakis(6-O-carboxymethyl-2,3-di-O-

methyl)cyclomaltoheptose buffer solution and lastly, the preparation of the racemic p-tolylsulfoximine sample which will be analyzed and separated.

The first sample, the Beckman standard solution kit, requires no sample preparation. After the column has been pre-treated with a 1% NaOH solution and rinsed thoroughly, the sample can be simply injected and separated using the provided boric acid buffer. The second set of solutions consists of a 4 mM sulfated cyclodextrin buffer and a synthesized p-tolylsulfoximine sample. A 4 mM sulfated cyclodextrin buffer was prepared by dissolving 75.0 mg of sulfated cyclodextrin (FW 1895.4 g/mol) in 10.0 ml of 0.1 M phosphate buffer (pH=2.5). The phosphate buffer was purchased from Biorad Inc. The methyl p-tolylsulfoximine (FW 353.5 g/mol) sample was prepared by dissolving 4.4 mg into 10.0 ml of 0.1 M phosphate buffer (pH=2.5). This gave us a 1.2 mM solution.

A series of different buffer and sample concentrations was prepared. We ran into some problems with "joule heating" which caused band broadening and I will discuss some of these issues in more detail in Chapter 3. The above mentioned solution ended up giving us baseline resolution between the two methyl p-tolylsulfoximine enantiomers.

For the single isomer functionalized cyclodextrin I also choose to prepare a 4 mM buffer solution by dissolving the cyclodextrin into 0.1 M phosphate buffer. The problem we encountered upon trying to separate the above mentioned sulfoximine solution is that at pH=2.5, the single isomer cyclodextrin is not completely ionized. This led to a dramatic reduction in resolution and the sulfoximine enantiomers could not be resolved.

2.7 Synthesis of Sulfoximine-Quinoline Derivative Species

In 1999, Kusumi et al.[27] reported the determination of the absolute configuration of chiral sulfoxides by derivatizing them to N-(α-methoxyphenylacetyl)sulfoximines. Based on their synthetic route we devised the following reaction scheme (Fig. 9);

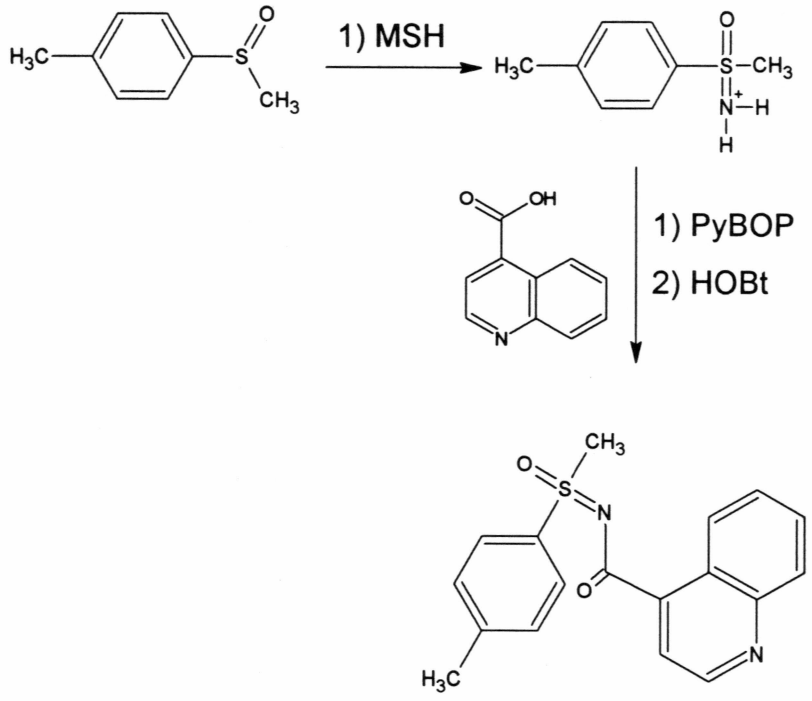


Fig. 9 Synthetic route to N-(4-quinolinecarboxylic)p-tolylsulfoximine

It is interesting to note that the N-hydroxybenzotriazole (HOBt) acts as a catalyst in this reaction and that the benzotriazol-1-yl-oxytripyrrolidinophosphonium

hexafluorophosphate (PyBOP, Fig. 10) acts as a coupling reagent between the carboxylic acid functionality and the amine functionality.

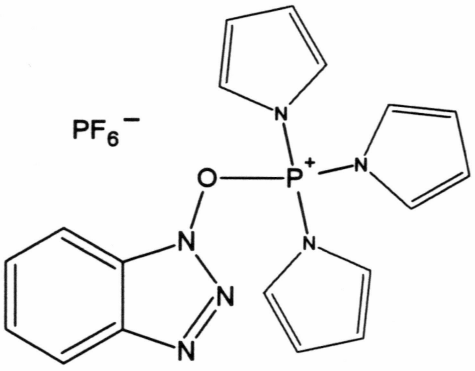


Fig. 10 Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)

The N-(4-quinolinecarboxylic)p-tolylsulfoximine was synthesized on a microscale by dissolving 37.0 mg (0.24 mmol) of methyl p-tolylsulfoximine in approximately 1.5 ml of dichloromethane. To this solution, 73.0 mg (0.34 mmol) of O-mesitylsulfonyl-hydroxylamine (MSH) was added. Kusumi et al.[27] also reported a 1:1.4 ration of sulfoxide-to-MSH, hence this slight excess of MSH. Immediately upon addition of the MSH, a cloudy white precipitate is observed. This mixture is stirred for 3 hours, at room temperature, under argon. After this period of stirring, 220 µl of pyridine, 76.2 mg (0.44 mmol) of 4-quinolinecarboxylic acid (once again we used the same molar ratios as did Kusumi[27]), 251.0 mg (0.48 mmol) of PyBOP and 66.0 mg (0.48 mmol) of HOBt, are added to the mixture, after which the mixture is stirred for an additional 3 hours at room

temperature. After the reaction time completed, the reaction mixture was diluted with approximately 5 ml of ethyl acetate. This organic layer was extracted twice with 10 ml of distilled water after which the ethyl acetate layer was isolated, dried (K_2SO_4) and pumped down to give a small amount of white solid. This solid was dissolved in $CDCl_3$ for NMR (NMR-9) and GC/MS characterization.

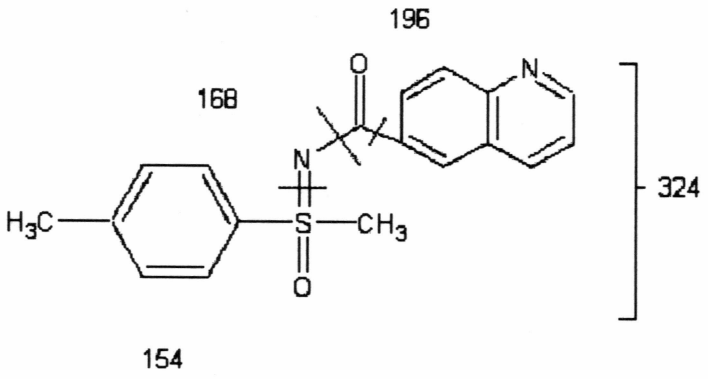


Fig. 11 GC/MS molecular weight fragments of N-(4-quinolinecarboxylic)p-tolylsulfoximine.

The molecular weight of the desired product, N-(4-quinolinecarboxylic)p-tolylsulfoximine, is 324 g/mol. GC/MS (DB-5 30m x 0.25 mm x 0.25 μm, temperature program: 90°C, 2 min., 15°C/min, 250°C, 5 min., injection temperature: 250°C) showed a molecular ion peak at 324 along with major fragments at 196, 170 (324-154), 156 (324-168) and 128. All these peaks correspond to fragments one would expect the desired

product to show (Fig. 11). NMR data also showed strong evidence that we were able to synthesize the above shown product (NMR-9).

We believe that this sulfoximine-quinoline derivative will make differentiating between the R and the S enantiomer of chiral sulfoxide much easier. One can imagine that both the quinoline aromatic functionality, as well as the p-tolyl group could interact with the cyclodextrin in the CE buffer. There is no obvious reason to presume that the derivatization reaction discriminates between one of the two sulfoxide enantiomers and hence we can assume that the enantiomeric excess is not affected by this derivatization. This however, is a claim which requires further investigation in future research. Obviously the reaction is of no use if the above claim is not valid. By derivitazing the sulfoximine with a heterocyclic species like 4-quinoline, we add four desirable characteristic to our sulfoximine species; 1) It becomes very UV absorbing so detection becomes possible for dialkylsulfoxides, which do not contain a phenyl group or some other UV absorbing functionality, 2) it provides a basic site for protonation thus allowing the derivative to be analyzed in a charged state, 3) one could use a fluorescence, acridine ring instead of 4-quinoline which means it can be detected by highly sensitive techniques like laser-induced fluorescence, lastly, 4) the large heterocyclic structure is obviously very hydrophobic (like the interior of the cyclodextrin), which leads to strong intracavity complexation between the derivatized sulfoximine and the cyclodextrin. This should lead to excellent resolution between enantiomers, although it might also lead to longer retention times and less time-efficient analyses.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Enantiomeric Separation of Methyl p-Tolylsulfoximine using Cyclodextrin Capillary Electrophoresis.

Before going into detail about our enantiomeric separation of racemic methyl p-tolylsulfoximine, it is important to go into more detail concerning the mechanisms of the cyclodextrin-analyte interactions. Some of the earlier work of looking into actual binding mechanisms was done by Martin Davies and Michael Deary[13] at the University of Northumbria at Newcastle, in the early 1990s. Their work focused on the calculation of binding constants between cyclodextrin species and a variety of sulfur-containing molecules. Much research has been done on the subject of cylodextrin chemistry but it was the research done by Davies and Deary, which shed some light on the actual driving forces behind the inclusion of guest species within the cyclodextin cavity. It has been shown that the inclusion of the guest species inside the cyclodextrin ring depends on several factors; a) Van der Waals interactions between the cyclodextrin and the guest species b) Hydrophobic interactions c) Release of high energy water or ring strain energy upon binding d) Hydrogen bonding between the guest species and the hydroxy groups (unless these groups have been changed to some other functionality), and lastly, e) effects of solvent surface tension. In the case of sulfoxides and sulfoximines, it is important to realize that the shape of the sulfur containing group is an important factor of both binding strength and orientation. Although my research has not really involved the subject of studying the actual interactions between the cyclodextrins and the guest species (in our

case the sulfoximines), the synthesis of the single isomer cyclodextrins does lay the foundation of a series of experiments which would further add to the knowledge we have of cyclodextrin-guest species interactions. After synthesizing the single isomer cyclodextrin, the first logical step to take was the comparison of the separation of methyl p-tolylsulfoximine by using either a single isomer cyclodextrin versus a mixed cyclodextrin, purchased from Aldrich Chemical Company. Recent research efforts, performed by Culha and Sepaniak[17] has shown that single isomer cyclodextrin capillary electrophoresis leads to narrowed peak separation and better resolution between enantiomers. My research will hopefully be the first step in developing a more simplified, single isomer chemical environment in which we can perform experiments which will clarify the degree of interaction between cyclodextrins, through hydrogen-bonding. Sepaniak investigated hydrogen-bonding interactions in mixed cyclodextrin systems but these systems are obviously much more complex and more difficult to investigate, due to the presence of a variety of differently substituted cyclodextrins.

The study of more simple, single isomer systems will be a very valuable undertaking, and it will help us in understanding hydrogen bonding in more complex, mixed systems.

As described in the experimental secion, I started by making sure the capillary electrophoresis system was running well, by injecting a test kit solution containing benzoic acid, p-hydroxybenzoic acid and p-hydroxyphenylacetic acid. The separation was performed at 11 kV using a 6 second injection onto our 50cm x 75μm bare fused silica column. The boric acid buffer used was also provided with the test kit.

Our separation looked good and was recorded to a file using the LABView program I developed. All data was recorded at 20 Hz, which lead to sufficient point-per-peak ratios. The electropherogram below shows the acquired separation of the test kit solution.

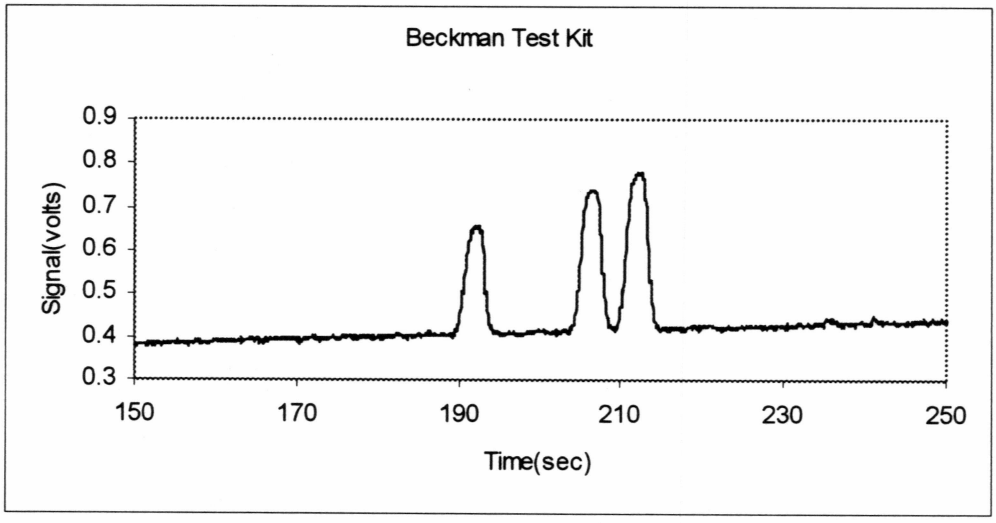


Fig. 12 CE separation of Beckman test kit solution

Once we established that the instrument was running correctly and the separation of the test kit was proven to be reproducible, we decided to attempt a separation of racemic p-tolylsulfoximine using a 1.6 mM chiral cyclodextrin selector solution. Once again, the buffer used was a 0.1 M phosphate buffer at pH=2.5. We attempted separation at 11 kV, 17 kV and 21 kV, but none of these settings resulted in enantiomeric separation, mainly because of joule heating, as seen in the plot on the next page.

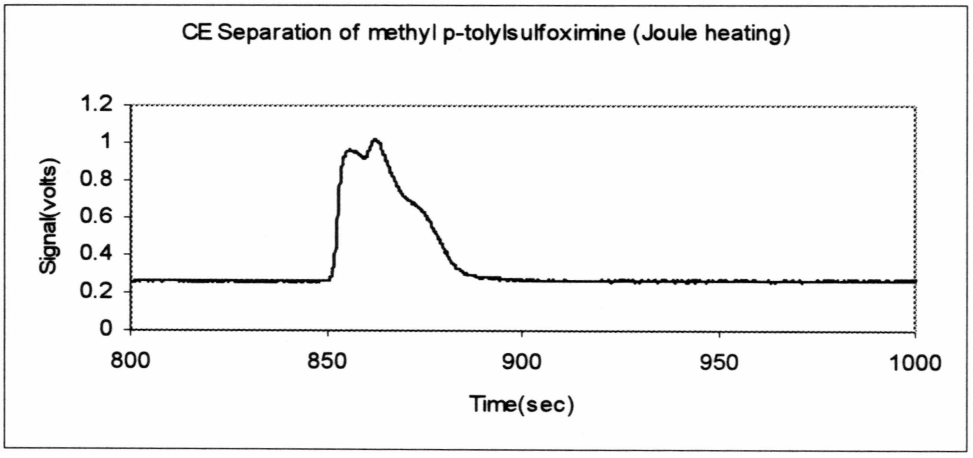


Fig. 13 CE separation of methyl p-tolylsulfoximine (Joule heating)

Our next experiment involved the introduction of a commercial, multi-substituted, sulfated cyclodextrin chiral selector, to the buffer solution. Our initial attempt consisted of 1.2 mM sulfoximine in 0.1 M phosphate buffer. The two buffer solutions contained 1.6 mM of sulfated cyclodextrin available from Aldrich. Initially we found that the capillary current was a little high so we reduced the buffer phosphate concentration to 0.08 molar. This also reduces the effect of "joule heating" which causes band broadening and loss of resolution. One can reduce the effects of joule heating by using smaller diameter capillary columns or reduced buffer concentration, which leads to lower current levels. Once we reduced our buffer concentration we saw improved peak shape and a slight improvement in resolution. This experiment showed that enantiomeric separation was possible but the R and S enantiomers were almost completely overlapped. We also increased the

cyclodextrin concentration to 2.5 M to give us an approximate 1:2 ratio of sulfoximine to cyclodextrin. This lead to somewhat better resolution.

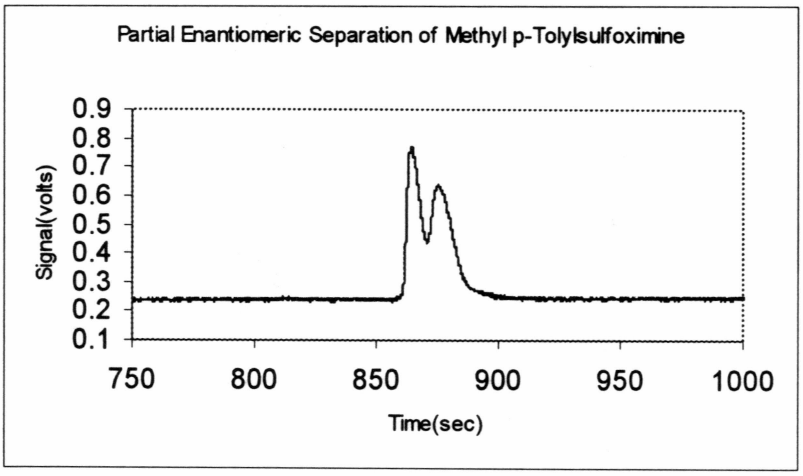


Fig. 14 CE separation of methyl p-tolylsulfoximine using 1:2 sulfoximine-cyclodextrin ratio

The best enantiomeric resolution was achieved by increasing the sulfated cyclodextrin concentration while maintaining a separation voltage of 17 kV (Fig. 15). We ran a sample of 1 mM sulfoximine in buffer, and the buffer solutions contained 4 mM of sulfated cyclodextrin. We achieved baseline resolved separation between the R and S enantiomers of the methyl p-tolylsulfoximine. Note that at this point of our research, we are not concerned about establishing the most effective, fastest separation. We are mainly interested in creating proper resolution between the two enantiomers. At no point did we perform actual integration or any form of quantitation. The goal was to develop the initial concepts in order to determine the enantiomeric excess in chiral compounds by showing that chiral selectors like cyclodextrins do indeed interact with the enantiomers in different ways, hence giving rise to improved enantiomeric separation.

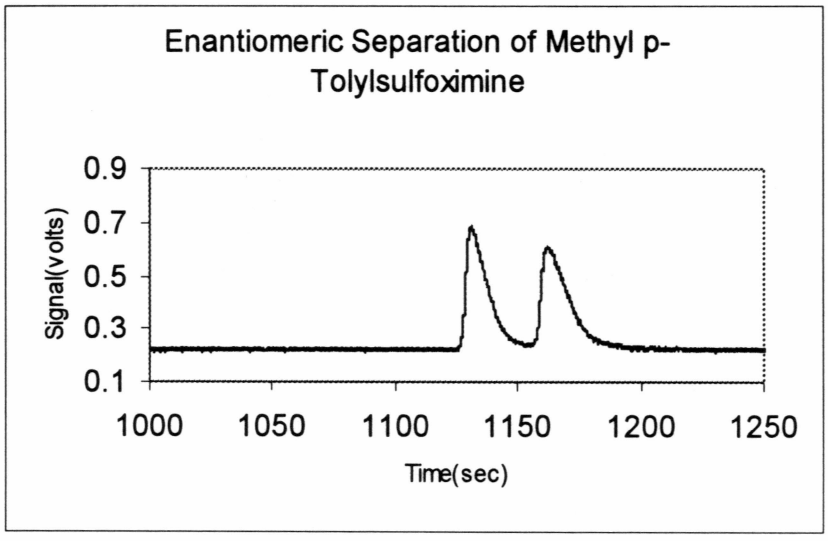


Fig. 15 CE separation of methyl p-tolylsulfoximine using 1:4 sulfoximine-cyclodextrin ratio

The last phase of the research was to study the difference between using a commercially available, sulfated cyclodextrin, consisting of several different substitutions and our synthesized, single isomer carboxylated cyclodextrin.

Initial results were not very positive; our single isomer cyclodextrin did not appear to lead to better separation or improved resolution between the sulfoximine enantiomers. We compared the commercial cyclodextrin, and our single isomer cyclodextrin by repeating the separation which I discussed on the previous page, where the commercial sulfated cyclodextrin chiral selector produced baseline separation between the two enantiomers, using the exact same solution concentrations and instrument settings, the single isomer gave rise to only one, unresolved peak. We have to realize that there really are two effects taking place at once; 1) the fact that our synthetic cyclodextrin consists of a singly substituted cyclodextrin whereas the commercial cyclodextrin contains more

several substitutions. The spectrum of insertion rates is more defined and less broad when using a single isomer cyclodextrin, this naturally leads to better resolution. Vigh refers to this effect as "parasitic complexation"[14] between the different isomers and warns us of the dangers of having a variety of complexation rates, when using a mixture of isomers. This is a guarantee for poor resolution. 2) Our synthetic cyclodextrin is structurally different, this might very well be the reason for poor separation. The carboxylated functionality might actually make it more difficult for the p-tolylsulfoximine to enter the cyclodextrin cavity. Ultimately we decided that the buffer pH was too low and that at pH=2.5, our single isomer was not ionized, while the commercial, sulfated cyclodextrin which is a strong acid will indeed be fully ionized. Indeed, this makes sense, one would expect a carboxylic group to have a pKa between 4-5. Obviously, the cyclodextrin should be ionized in order to achieve better separation in a capillary electrophoretic system.

It is difficult to say which effects are predominantly at work between the cyclodextrin and guest species interactions, there is a steric effect which relates to ease of insertion and the rates associated with this insertion of the guest species into the cyclodextrin cavity. By altering the shape of the cyclodextrin cavity, as with our carboxylic functionality, we change the cyclodextrin steric environment. Another important effect is the electronic effect of having a different charge distribution on the cyclodextrin molecule. It is very difficult to make any specific declarations concerning which effect contributes the strongest in our example, and I believe that a better comparison might be between the butylsultone functionalized cyclodextrin and the commercially available sulfated cyclodextrin, since these molecules do not differ

significantly electronically but they do differ significantly from a steric, molecular shape standpoint. This investigation should be included in future studies.

3.2 Indirect Detection of Single Isomer Cyclodextrins

After the synthesis of the single isomer cyclodextrin it becomes necessary to think of some method to determine if the synthetic product truly is a single isomer. The most common way of doing this, is by using indirect detection, using capillary electrophoresis. This method of analyses is based on the reduced UV absorption, as compared to the buffer background, when the sample passes by the detector. Hence, in indirect detection, our peaks are negative (downward orientation). Since the buffer background contains a molecular species which absorbs UV light, it is very common to experience additional levels of background noise. So interestingly enough, our single isomer cyclodextrin, which will be used to improve capillary electrophoresis experiments, will first need to be analyzed by CE, itself. Some of the more common choices for a background buffer are either phthalic acid background buffers or p-toluenesulfonic acid background buffers. We experimented with a phthalic acid buffer (20 mM) which was brought up to pH=8.5 using TRIS base. But this resulted in a very noisy background and based on time limitations we decided to pursue a different route. We decided to use p-toluenesulfonic acid instead. This has been used very successfully by Vigh[28] in separating functionalized cyclodextrin isomers. We made a 20 mM buffer solution of p-toluenesulfonic acid which was brought up to pH=8 using TRIS base. Two sample solutions were made up, one 1 mM single isomer carboxylated cyclodextrin in buffer solution and one 20 mM single isomer

carboxylated cyclodextrin in buffer solution. The system was run at 25 kV and we used a wavelength of 214 nm. The 1 mM cyclodextrin sample proved to be much too dilute and we were not able to detect the sample above the background. The 20 mM sample gave three, relatively weak, signals at the retention times which could correspond to 6-, and 7-substituted carboxylated cyclodextrin, in addition to an impurity peak which appeared on other occasions, while looking at butylsulfated cyclodextrin. We based this on the Vigh[39] article in which he analyzes a functionalized cyclodextrin which has the same –7 negative charge and the approximate same molecular mass, hence one would expect very similar retention times, under the same capillary electrophoresis conditions. As in Vigh's experiments, we also see a large positive peak for the Na^+ associated with the cyclodextrin.

Based on our findings, our carboxylated cyclodextrin is not truly a singly, substituted isomer (7-substitution), but instead there appear to be two individual substitutions present. The electropherogram below (Fig. 16) is our indirect detection experiment for the analysis of the heptakis(6-O-carboxymethyl-2,3-dimethyl)-cyclomaltoheptose.

The first two peaks are 6-, and 7-carboxylate substituded cyclodextrin, while the third peak appears to be some impurity which is later observed in different indirect detection experiments.

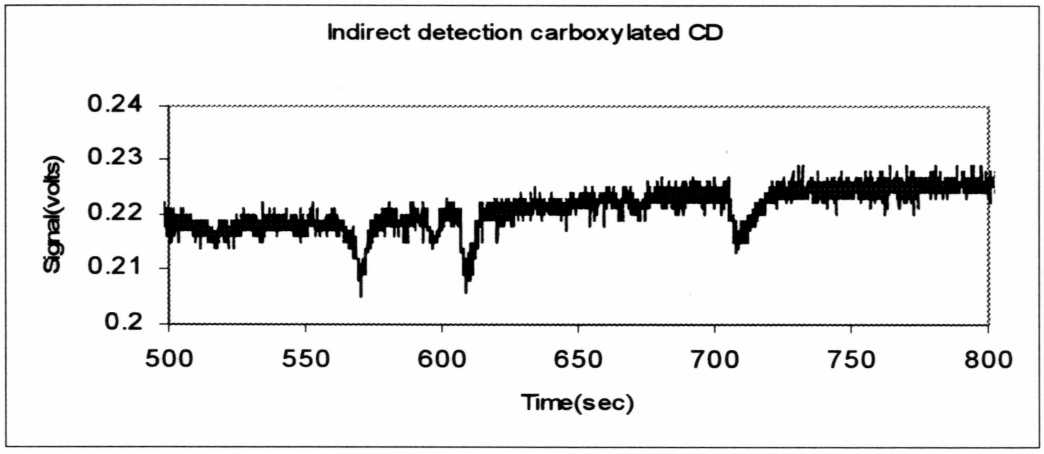


Fig. 16 Indirect detection of heptakis(6-O-carboxymethyl-2,3-dimethyl)cyclo-maltoheptose

We did have some very exciting findings with the butylsultone functionalized cyclodextrin. We used a 20 mM p-toluenesulfonic acid buffer as a background buffer. The instrument was run at 20 kV, using a 1 second injection time. The following indirect detection electropherogram was observed.

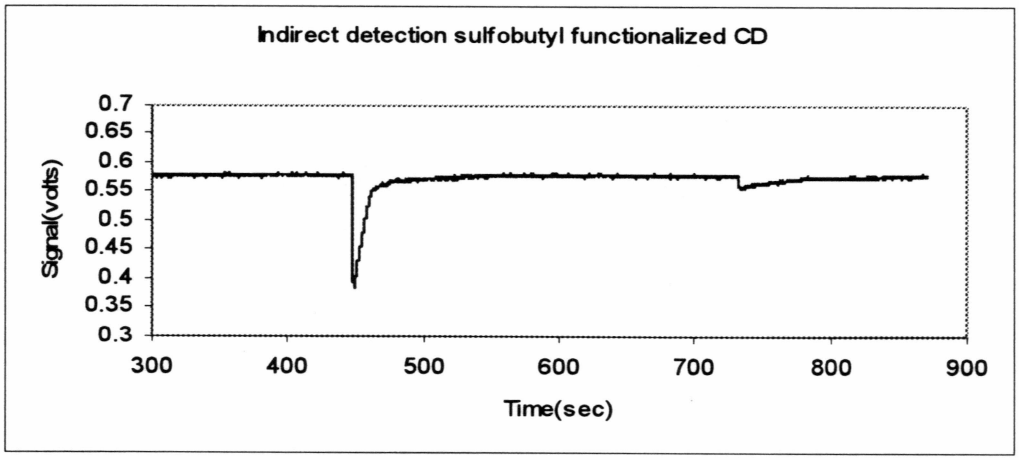


Fig. 17 Indirect detection of butylsultone functionalized cyclodextrin

We learn from this experiment that this reaction, using very long reaction times, will give predominantly a singly substituted product. The peak to the right of the main peak is most likely due to the ring-opened, unreacted, anionic 1,4-butane sultone. Since we are running normal polarity CE, it makes sense that anions with larger charge-to-mass ratios would have longer retention times.

CHAPTER 4. CONCLUSIONS

This research project has been a wonderful and intellectually challenging experience. Having been Dr. Green's first graduate student at the University of Alaska Fairbanks, it was my goal to lay down a foundation of research on which future students can build. This goal was achieved by demonstrating a number of interesting concepts; 1) We were able to effectively convert sulfoxides into sulfoximine species, using O-mesitylsulfonyl- hydroxylamine 2) We have shown that there are a variety of synthetic routes which can be used to synthesize novel, functionalized cyclodextrins. We were able to synthesize heptakis(6-O-carboxymethyl-2,3-dimethyl)cyclomaltoheptose, and although our indirect detection experiment suggests that we have a mix of substitution products, I do believe that this problem can be avoided in future research efforts by focussing on using preparative HPLC to separate the different substitution products. This method is used by Vigh et al. and it does seem very reasonable that a simple silica column separation does not have enough theoretical plates to separate 5-, 6-, and 7-substituted cyclodextrins 3) We have shown, using commercially available, mixed isomer sulfated cyclodextrin as a chiral selector, that sulfoximine enantiomers can be separated from each other using cyclodextrin capillary electrophoresis 4) We were able to synthesize a truly novel quinoline-sulfoxide derivative which we were able to identify by NMR and GC-MS. This derivatization technique deserves further attention in future research efforts by the Green group. We have shown that the chemistry works and now it becomes important

to show that stereochemistry is retained in this reaction, and to study its separation behavior in cyclodextrin capillary electrophoresis experiments.

We have shown that there are a variety of synthetic routes which can be used in order to create novel, functionalized cyclodextrins. As with virtually all synthetic organic research, the possibilities are virtually endless. I think that it would be very interesting, in future research efforts, to focus on learning more about the actual cyclodextrin-guest species interactions and to then, develop specific single isomer functionalized cyclodextrins which meet very specific criteria. By introducing a specific functionality, in our case a carboxylic group, one can change the interaction effects between the cyclodextrin and the guest species, the steric and electronic effects involved and the size and shape of the cyclodextrin cavity.

We were also able to synthesize a butylsultone functionalized cyclodextrin, though this product is still in the identification stage. The final step of this reaction in which the butylsultone functionality is introduced, takes place in an aqueous phase and it has proven to be very difficult to extract these cyclodextrins into an organic phase. Cyclodextrins act much like soaps, in that they contain a very hydrophobic core while at the same time contain these easily ionized, highly polar functionalities. This leads to great difficulties in extractions and purifications. Choosing appropriate organic solvents becomes quite difficult.

When creating these cyclodextrins it is very important to realize that both steric effects as well as electronic effects are important in the nature of cyclodextrin-guest species interactions. I believe that it might be interesting to focus future investigations on

the steric interactions. From a chemical standpoint, I think it might be possible that the guest species insert deeper into cyclodextrin cavities with extended, hydrophobic carbon chains hanging from the bottom of the cyclodextrin. The figure below attempts to show that in the carboxylated cyclodextrin we mainly deal with relatively simple, electronic interactions between cyclodextrin and guest species but in the case of the butylsultone functionalized cyclodextrin I believe that, in a sense, we have extended and elongated the hydrophobic core of the cyclodextrin, which leads to possible deeper insertion of the guest species into the cyclodextrin. This in turn should lead to more defined insertion complexation rates between the cyclodextrin and the analyte, and hence increased the enantiomeric resolution.

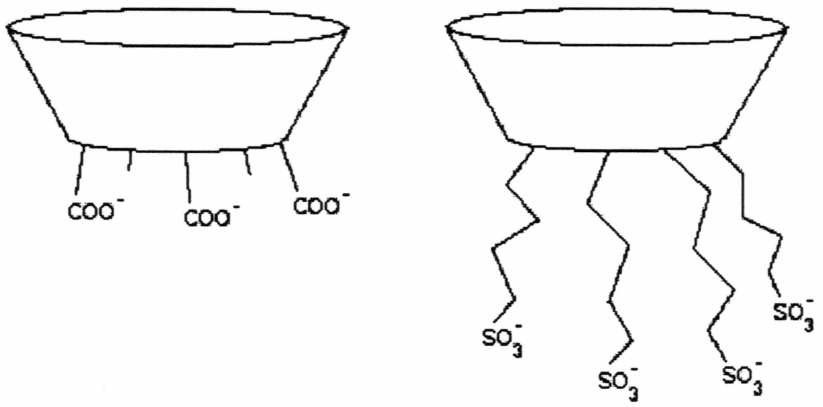


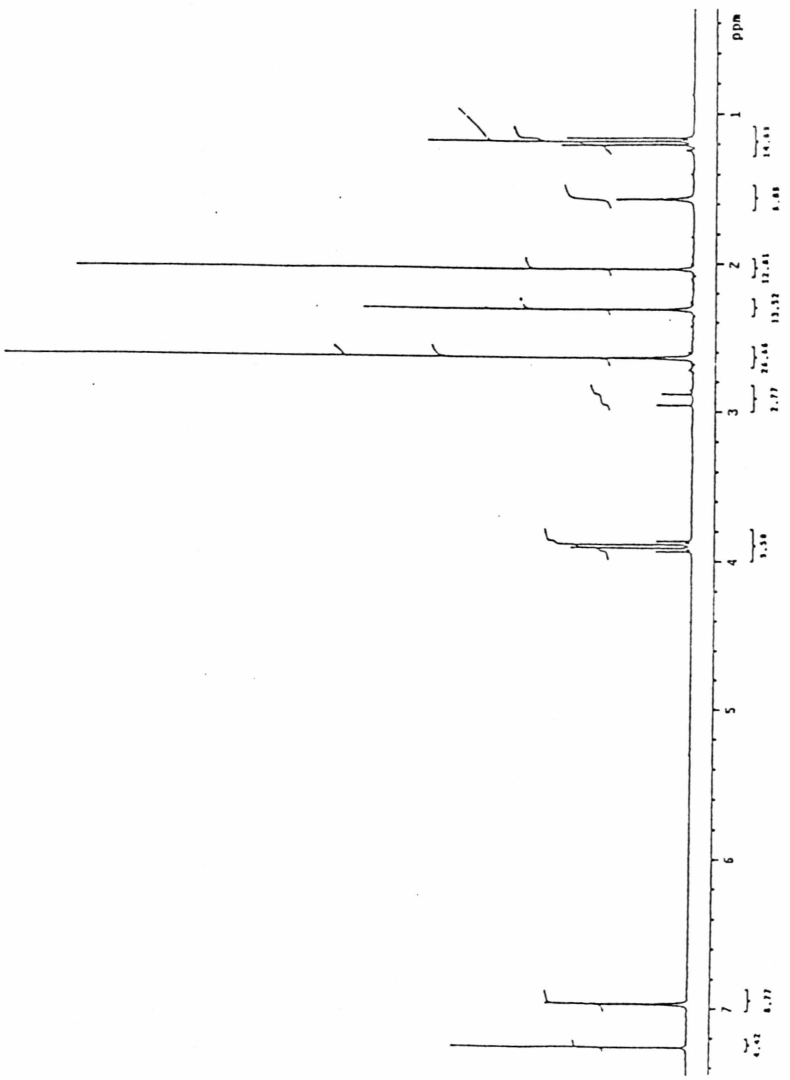
Fig. 18 Carboxylated versus butylsulfonated cyclodextrin

Note that in the figure above there would be 5-, 6-, and 7-substitution, but in order to retain clarity only a few substitutions are shown.

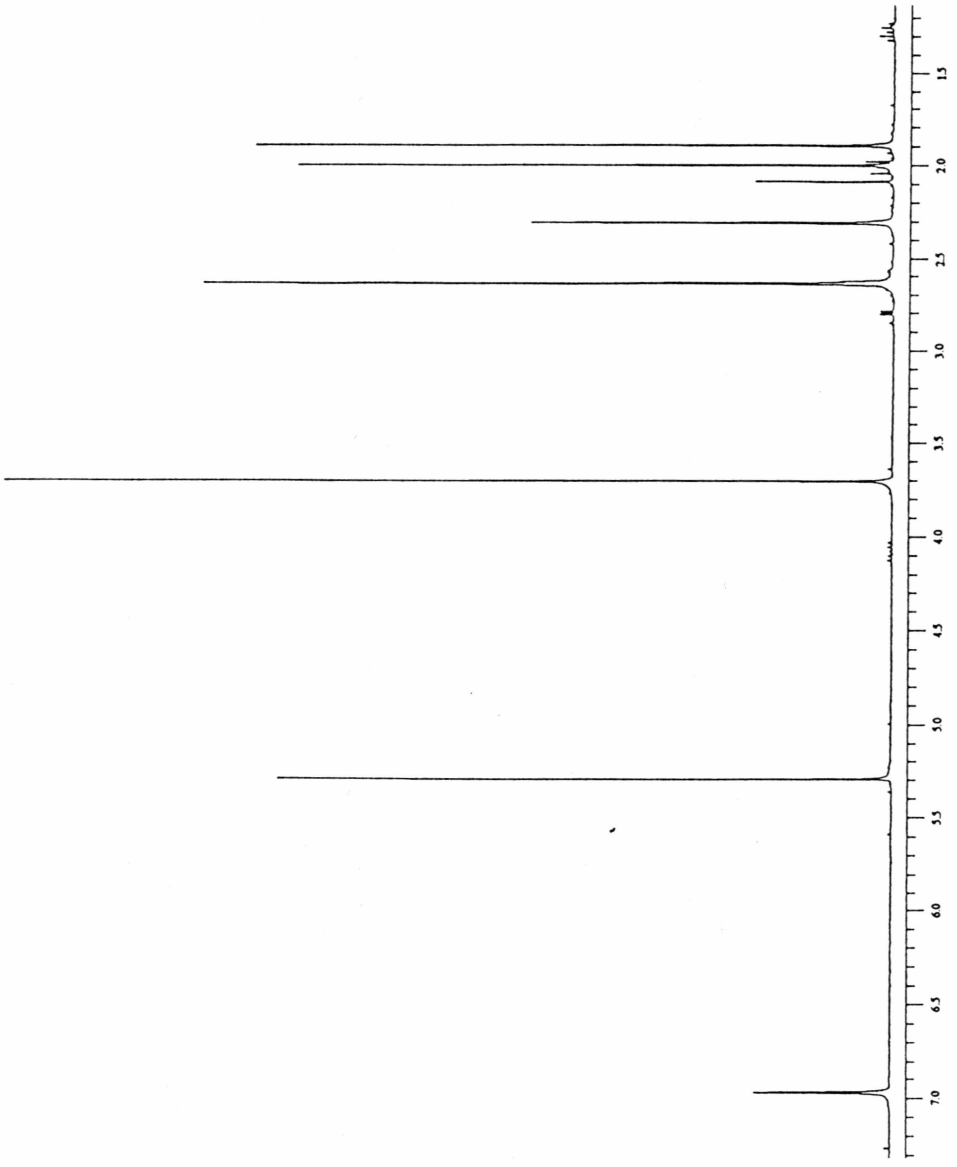
Lastly, there are some interesting experiments which might be done using indirect detection with capillary electrophoresis. One such experiment would be to monitor reaction progress and kinetics of different functionalization reactions of the cyclodextrin molecule. Dr. Green and I both agree that in hindsight, the butylsultone functionalized cyclodextrin is of much greater interest, due to its steric characteristics as compared to the carboxylated cyclodextrin. The future focus will most likely be on this family of chemical species.

APPENDIX 1: Nuclear Magnetic Resonance Spectra

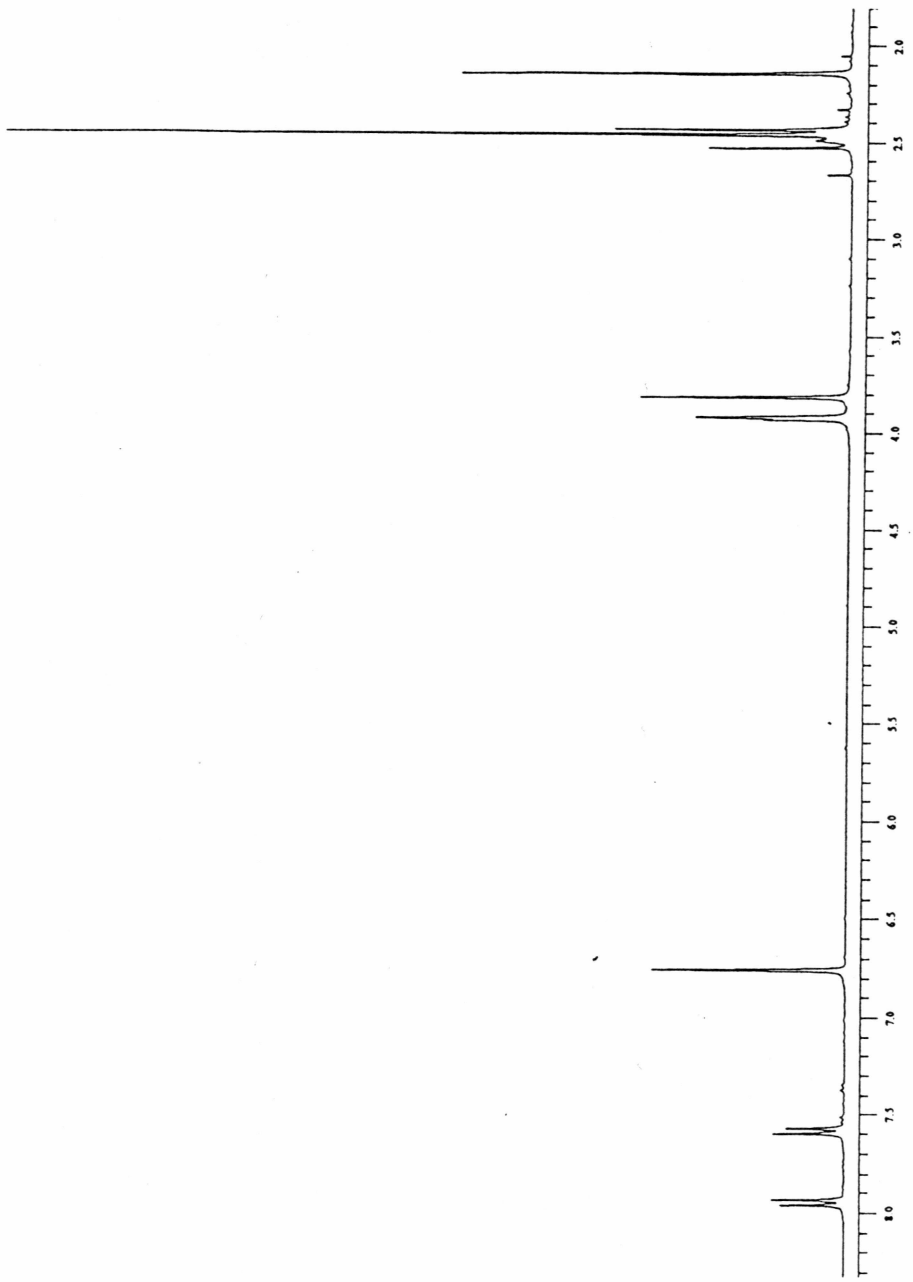
NMR-1 Ethyl O-(mesitylsulfonyl)acetohydroxamate



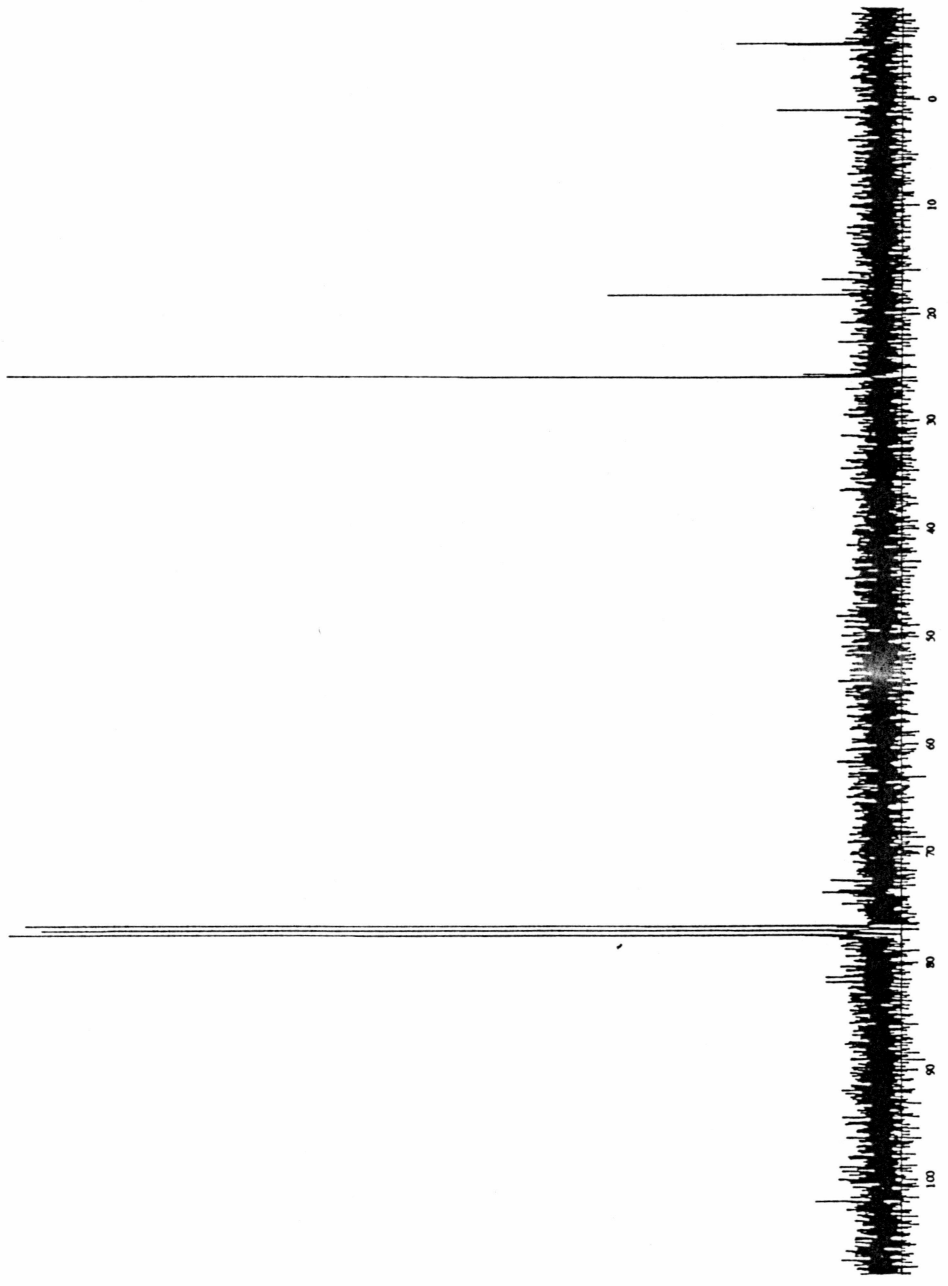
NMR-2 Mesitylsulfonylhydroxylamine



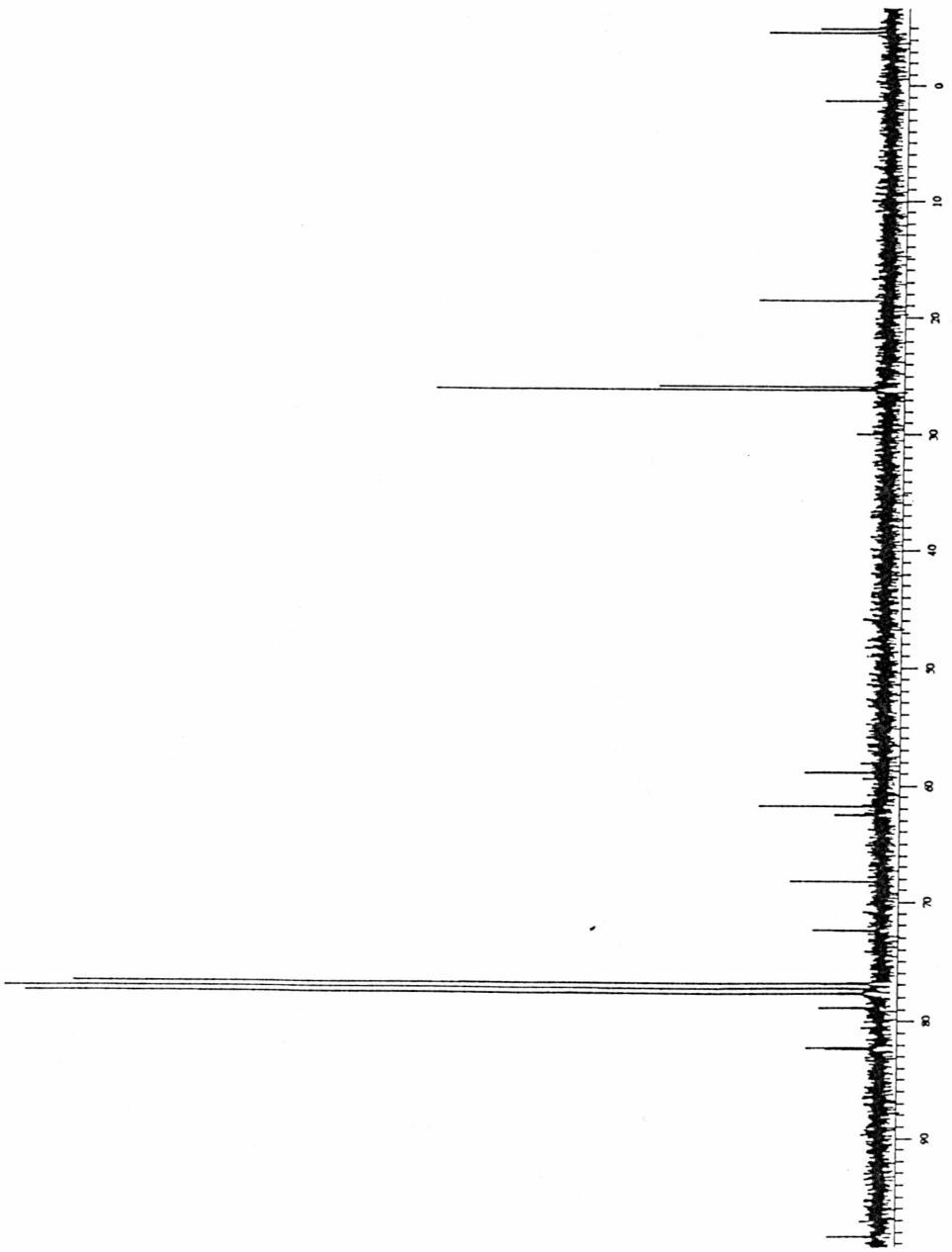
NMR-3 p-Tolylsulfoximine



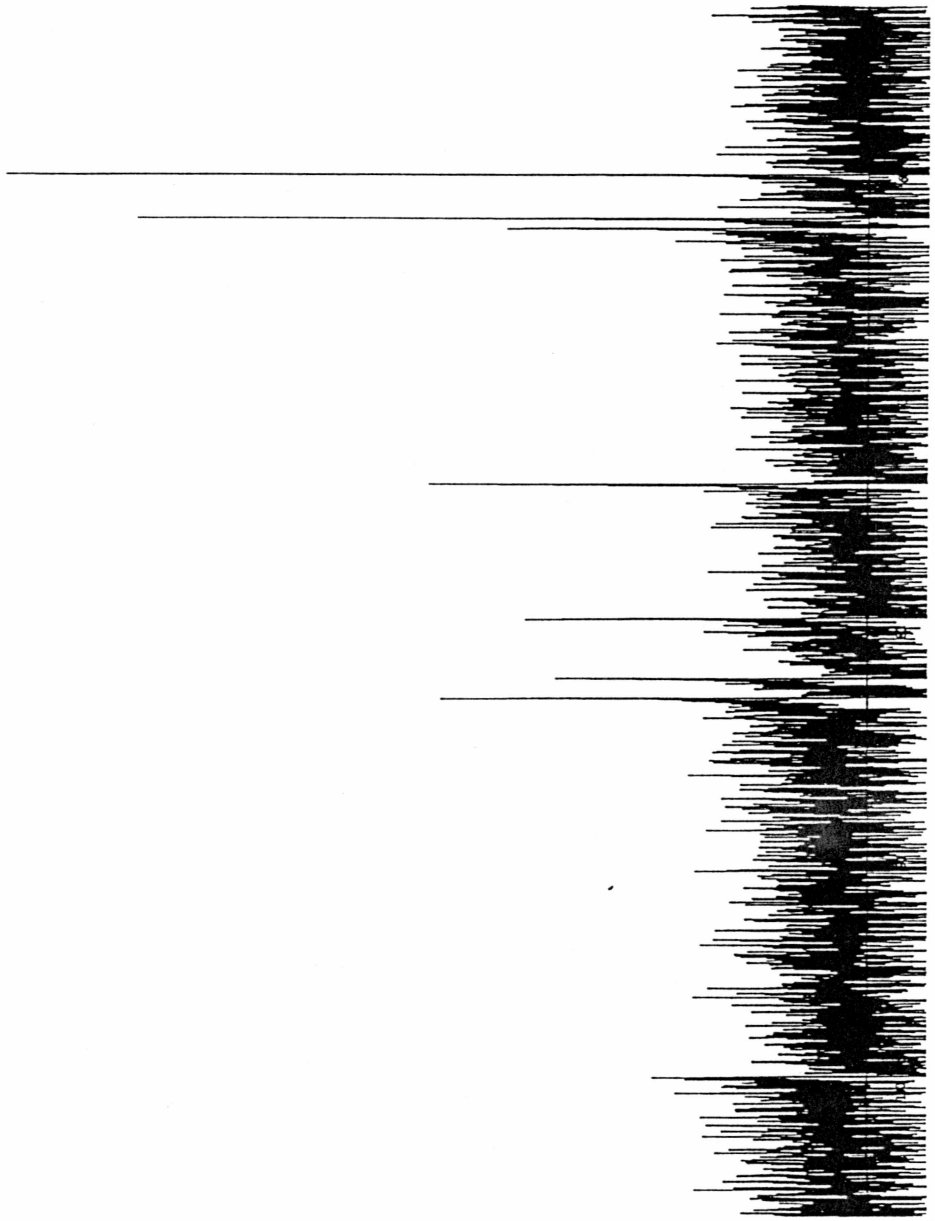
NMR-4 Heptakis(6-O-tert-butyldimethylsilyl)cyclomaltoheptose



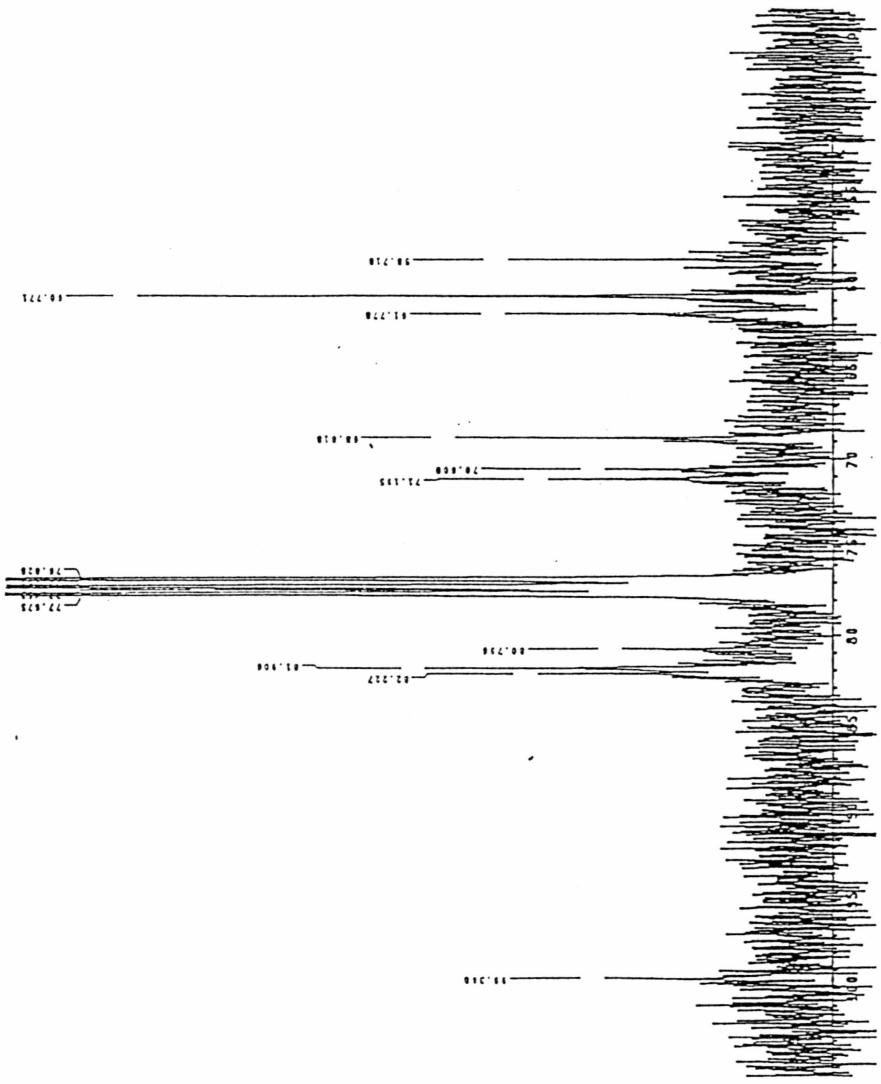
NMR-5 Heptakis(6-O-tert-butyldimethylsilyl-2,3-di-O-methyl)cyclomaltoheptose



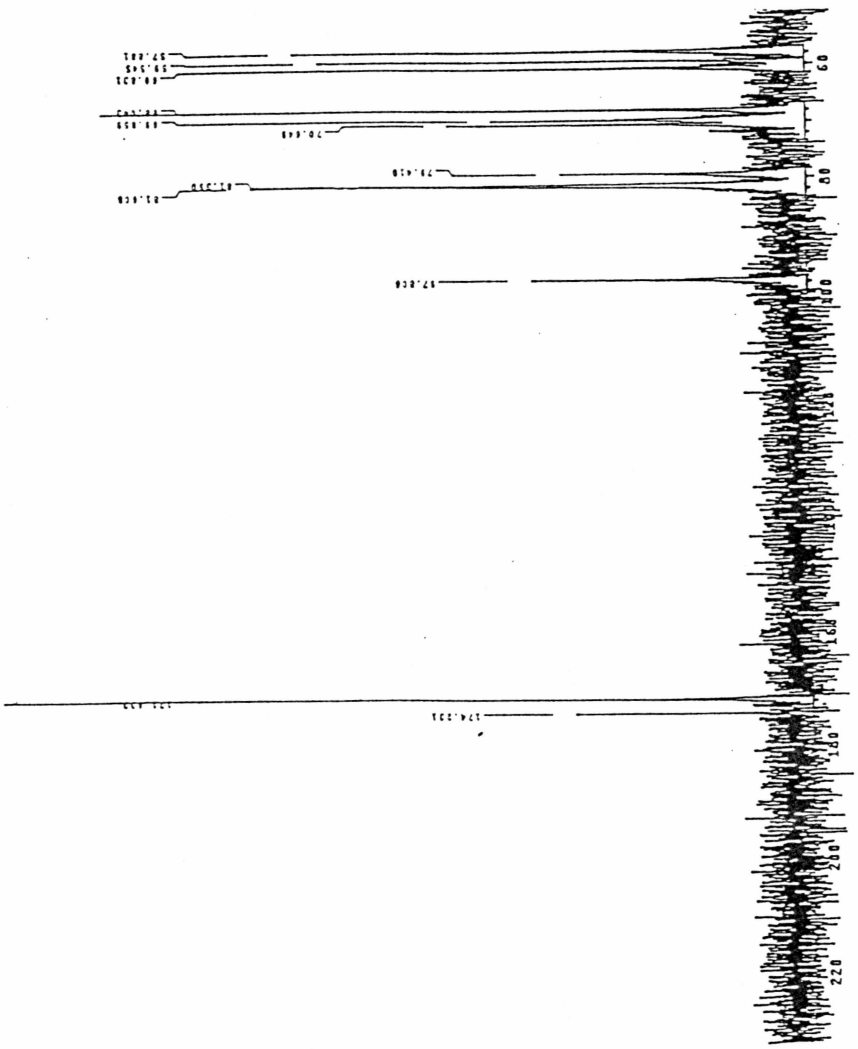
NMR-6 Heptakis(2,3-di-O-methyl)cyclomaltoheptose



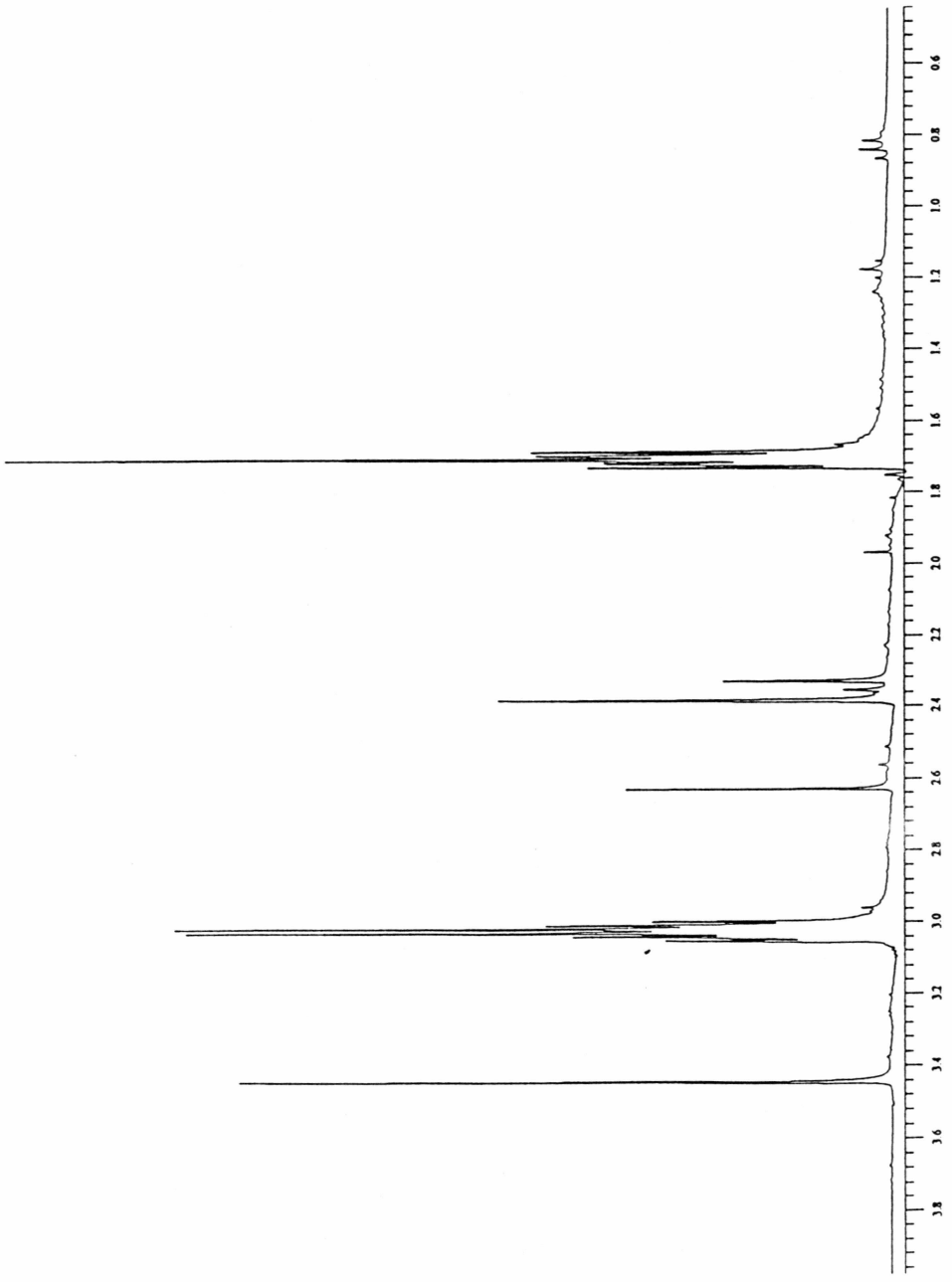
NMR-7 Heptakis(6-O-ethoxycarbonylmethyl-2,3-di-O-methyl)cyclomaltoheptose



NMR-8 Heptakis(6-O-carboxymethyl-2,3-di-O-methyl)cyclomaltoheptose



NMR-9 N-(4-quinolinecarboxylic)p-tolylsulfoximine



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